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(71) Applicant *(for all designated States except US)*: YALE UNIVERSITY [US/US]; Room 210, 155 Whitney Avenue, P.O. Box 208336, New Haven, CT 06520-8336 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: DIB-HAJJ, Sulayman [US/US]; Room 210, 155 Whitney Avenue, P.O. Box 208336, New Haven, CT 06520-8336 (US). WAXMAN, Stephen, G. [US/US]; 155 Whitney Avenue, P.O. Box 208336, New Haven, CT 06520-8336 (US).

(74) Agent: TUSCAN, Michael, S.; Morgan, Lewis & Bockius LLP, 1800 M. Street, N.W., Washington, DC 20036-5869 (US).

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(54) Title: MODULATION OF HUMAN SODIUM CHANNELS IN DORSAL ROOT GANGLIA

(57) Abstract: A novel human tetrodotoxin resistant sodium channel is described, along with isolated nucleic acid molecules that encode this channel. Methods for identifying agents that modulate the Na^+ current through the channel are provided, as well as related therapeutic and diagnostic methods.

MODULATION OF HUMAN SODIUM CHANNELS IN DORSAL ROOT GANGLIA

FIELD OF THE INVENTION

The present invention relates to a novel human tetrodotoxin-resistant sodium channel and related nucleotides, as well as screening assays for identifying agents useful in treating acute or chronic pain or other hyperexcitability states. This application is related to U.S. Provisional Application 60/072,990, filed January 29, 1998; U.S. Provisional Application 60/109,402 filed November 20, 1998; U.S. Provisional Application 60/109,666 filed on November 20, 1998 ; PCT International Application PCT/US99/02008 filed January 29, 1999 and U.S. Patent Application 09/354,147 filed July 16, 1999, all of which are herein incorporated by reference in their entirety.

BACKGROUND

A. Sodium Channels

Voltage-gated sodium channels are a class of specialized protein molecules that act as molecular batteries permitting excitable cells (neurons and muscle fibers) to produce and propagate electrical impulses. Voltage-gated Na^+ channels from rat brain are composed of three subunits, the pore-forming a subunit (260 KDa) and two auxillary subunits, b1 (36 KDa) and b2 (33 KDa) that may modulate the properties of the a-subunit; the a subunit is sufficient to form a functional channel that generates a Na current flow across the membrane (Catterall, (1993) Trends Neurosci. 16, 500-506; Isom *et al.*, (1994) Neuron 12, 1183-1194). Nine distinct a subunits have been identified in vertebrates and are encoded by members of an expanding gene family (Goldin (1995) Handbook of receptors and channels (North, editor) CRC Press; Akopian *et al.*, (1996) Nature 379, 257-262; Akopian *et al.*, (1997) FEBS Lett. 400, 183-187; Sangameswaran *et al.*, (1996) J. Biol. Chem. 271, 5953-5956) and respective orthologues of a number of them have been cloned from various mammalian species including humans. Specific a subunits are expressed in a tissue- and developmentally-specific manner (Beckh *et al.*, (1989) EMBO J. 8, 3611-3616; Mandel, (1992) J. Membr. Biol. 125, 193-205).

Aberrant expression patterns or mutations of voltage-gated sodium channel a-subunits underlie a number of human and animal disorders (Roden & George, (1997) Am. J. Physiol. 273, H511-H525; Ptacek, (1997) Neuromuscul. Disord. 7, 250-255; Cannon, (1997) Neuromuscul. Disord. 7; 241-249; Cannon, (1996) Trends Neurosci. 19, 3-10); Rizzo *et al.*, (1996) Eur. Neurol. 36, 3-12).

Voltage-gated sodium channel a-subunits consist of four domains (D1-4) of varying internal homology but of similar predicted structure, connected by three intracellular loops (L1-3). The four domains fold to form a channel that opens to both the cytoplasm and the extracellular space via a pore. The pore opens and closes depending upon the physiological state of the cell membrane.

Each domain consists of six transmembrane segments (S1-6) that allow the protein to weave through the membrane with intra- and extracellular linkers. The linkers of S5-S6 segments of the four domains contain sequences that line the pore of the channel, and a highly conserved subset of amino acids that acts as a filter to selectively allow sodium ions to traverse the channel pore into the cytoplasm, thus generating an electric current. The amphiphatic S4 segment, in each of the four domains, rich in basic residues repeated every third amino acid, acts as a voltage sensor and undergoes a conformational change as a result of the change in the voltage difference across the cell membrane. This in turn triggers the conformational change of the protein to open its pore to the extracellular Na^+ ion gradient.

In most of the known voltage-gated sodium channel a-subunits the channels close and change into an inoperable state quickly (inactivate) within a few milliseconds after opening of the pore (activation); SNS-type channels, on the other hand, inactivate slowly and require a greater voltage change to activate. L3, the loop that links domains D3 and D4, contains a tripeptide which acts as an intracellular plug that closes the pore after activation, thus inducing the channel to enter the inactive state. After inactivation, these channels further undergo conformational change to restore their resting state and become available for activation. This period is referred to as recovery from inactivation (repriming). Different channels reprime at different rates, and repriming in SNS is relatively rapid.

Based on amino acid similarities, the voltage-gated sodium channel family has been further subdivided into two subfamilies (Felipe *et al.*, (1994) J. Biol. Chem. 269, 30125-30131). Eight of the nine cloned channels belong to subfamily 1. They share many structural features, particularly in their S4 transmembrane segments. However, some of them have been shown to have distinct kinetic properties of inactivation and repriming. Only a single channel of subfamily 2, also referred to as atypical channels, has been identified in human, rat and mouse tissues. This subfamily is primarily characterized by reduced numbers of basic residues in its S4 segments, and thus is predicted to have different voltage-dependence compared to subfamily 1. The physiological function of subfamily 2 channels is currently unknown because its electrophysiological properties have not yet been elucidated.

The blocking of voltage-gated sodium channels by tetrodotoxin, a neurotoxin, has served to functionally classify these channels into sensitive (TTX-S) and resistant (TTX-R) phenotypes. Two mammalian TTX-R channels have so far been identified, one specific to the cardiac muscle and to very limited areas of the central nervous system (CNS) and the second, SNS, is restricted to peripheral neurons (PNS) of the dorsal root ganglia (DRG) and trigeminal ganglia. Specific amino acid residues that confer resistance or sensitivity to TTX have been localized to the ion selectivity filter of the channel pore. The SNS channel is also described in International Patent Application WO 97/01577.

B. <u>Role of Sodium Channels in Disease States</u>

Because different Na^+ channel a-subunit isotypes exhibit different kinetics and voltage-dependence, the firing properties of excitable cells depend on the precise mixture of channel types that they express. Mutants of the cardiac and skeletal muscle a-subunit have been shown to cause a number of muscle disorders. Some examples are as follows: A change of a single basic amino acid residue in the S4 of the skeletal muscle channel is sufficient to change the kinetic properties of this channel and induce a disease state in many patients. A tripeptide deletion in L3 of the cardiac channel, proximal to the inactivation gate, induces a cardiac disorder called Long QT syndrome. A single amino acid change in the S5-S6 linker of

domain 1 of Scn8a, the region lining the pore of the channel, causes the mouse mutant "jolting". The total loss of this channel by a different mutation causes motor end plate "med" disease in mice. This mutation is characterized by loss of motor neuron stimulation of the innervated muscle.

C. **Sodium Channels and Pain**

Axonal injury (injury to nerve fibers, also called axons) can produce chronic pain (termed neuropathic pain). A number of studies have demonstrated altered excitability of the neuronal cell body and dendrites after axonal injury (Eccles *et al.*, (1958) J. Physiol. 143: 11-40; Gallego *et al.*, (1987) J. Physiol. (Lond) 391, 39-56; Kuno & Llinas, (1970) J. Physiol. (Lond.) 210, 807-821), and there is evidence for a change in Na^+ channel density over the neuronal cell body and dendrites following axonal injury (Dodge & Cooley, (1973) IBM J. Res. Dev. 17, 219-229; Titmus & Faber (1986) J. Neurophysiol. 55, 1440-1454; Sernagor *et al.*, (1986) Proc. Natl. Acad. Sci. USA 83, 7966-7970). The expression of abnormal mixtures of different types of sodium channels in a neuronal cell can also lead to abnormal firing (Rizzo *et al.*, (1996) Eur. Neurol. 36, 3-12), and can contribute to hyperexcitability, paresthesia or pain.

Recent studies on rat sensory DRG neurons have demonstrated a dramatic change in the expression profile of TTX-R and TTX-S currents and in a number of mRNA transcripts that could encode the channels responsible for these currents in DRG neurons following various insults (Rizzo *et al.*, (1995) Neurobiol. Dis. 2: 87-96; Cummins *et al.*, (1997) J. Neurophysiol. 17, 3503-3514; Dib-Hajj *et al.*, (1996) Proc. Natl. Acad. Sci. USA 93, 14950-14954). For example, it has been shown an attenuation of the slowly inactivating, TTX-R current and simultaneous enhancement of the rapidly inactivating, TTX-S Na^+ currents in identified sensory cutaneous afferent neurons following axotomy (Rizzo *et al.*, (1995) Neurobiol. Dis. 2, 87-96). A loss of TTX-S, slowly repriming current and TTX-R current and a gain in TTX-S, rapidly repriming current in nociceptive (pain) neurons following axotomy (Cummins & Waxman (1997) J. Neurophysiol. 17, 3503-3514), down-regulation of SNS transcripts and a simultaneous up-regulation of α-III Transcripts has also been shown

(Dib-Hajj *et al.*, (1996) Proc. Natl. Acad. Sci. USA 93, 14950-14954). Also associated with axotomy is a moderate elevation in the levels of aI and aII mRNAs (Waxman *et al.*, (1994) J. Neurophysiol. 72, 466-470). These changes in the sodium channel profile appear to contribute to abnormal firing that underlies neuropathic pain that patients suffer following axonal injury.

Inflammation, which is also associated with pain (termed inflammatory pain), also causes alteration in the sodium current profile in nociceptive DRG neurons. Inflammatory modulators up-regulate TTX-R current in small C-type nociceptive DRG neurons in culture (Gold *et al.*, (1996) Proc. Natl. Acad. Sci. USA 93, 1108-1112; England *et al.*, (1996) J. Physiol. 495, 429-440). The rapid action of these modulators suggests that their action include posttranslational modification of existing TTX-R channels. It has now been determined that inflammation also increases a TTX-R Na^+ current and up-regulates SNS transcripts in C-type DRG neurons (Tanaka *et al.*, (1998) Neuroreport. 9, 967-972). This data suggests that changes in the sodium current profile contribute to inflammation evoked-pain.

D. <u>Therapies for Chronic Pain</u>:

A variety of classes of drugs (anticonvulsants such as phenytoin and carbamazepine; anti-arrhythmics such as mexitine; local anesthetics such as lidocaine) act on Na^+ channels. Since the various Na^+ channels produce sodium currents with different properties, selective blockade or activation (or other modulation) of specific channel subtypes is expected to be of significant therapeutic value. Moreover, the selective expression of certain a-subunit isoforms (PN1, SNS, NaN) in specific types of neurons provides a means for selectively altering their behavior.

Nociceptive neurons of the DRG are the major source of the PNS TTX-R Na^+ current. Thus, the Na^+ channels producing TTX-R currents provide a relatively specific target for the manipulation of pain-producing neurons. The molecular structure of one TTX-R channel in these DRG neurons, SNS, has been identified but, prior to our research, it has not been determined whether there are other TTX-R channels in these neurons. If such channels could be identified, they would be ideal candidates as target molecules that are preferentially expressed in nociceptive neurons, and whose modulation would attenuate pain transmission.

SUMMARY OF THE INVENTION

The present invention includes an isolated nucleic acid which encodes a voltage gated Na^+ channel that is preferentially expressed in dorsal root ganglia or trigeminal ganglia (the NaN channel). (In our preceding U.S. Provisional Application 60/072,990, this NaN channel was referred to by its previous name "NaX"). In a preferred embodiment, the isolated nucleic acid comprises the sequence shown in Figure 1 (SEQ ID NO: 1), Figure 7A (SEQ ID NO: 4), Figure 8A (SEQ ID NO: 6), Figure 11A (SEQ ID NO: 41), allelic variants of said sequences or nucleic acids that hybridize to the foregoing sequences under stringent conditions.

In another embodiment, the invention includes an expression vector comprising an isolated nucleic acid which encodes the voltage gated Na^+ channel that is preferentially expressed in dorsal root ganglia or trigeminal ganglia either alone or with appropriate regulatory and expression control elements. In a preferred embodiment, the expression vector comprises an isolated nucleic acid having the sequence shown in Figure 1 (SEQ ID NO: 1), Figure 7A (SEQ ID NO: 4), Figure 8A (SEQ ID NO: 6), Figure 11A (SEQ ID NO: 41), allelic variants of said sequences or nucleic acids that hybridize to the foregoing sequences under stringent conditions.

The present invention further includes a host cell transformed with an expression vector comprising an isolated nucleic acid which encodes a voltage gated Na^+ channel that is preferentially expressed in dorsal root ganglia or trigeminal ganglia with appropriate regulatory and expression control elements. In a preferred embodiment, the expression vector comprises an isolated nucleic acid having the sequence shown in Figure 1 (SEQ ID NO: 1), Figure 7A (SEQ ID NO: 4), Figure 8A (SEQ ID NO: 6), Figure 11A (SEQ ID NO: 41), allelic variants of said sequences or nucleic acids that hybridize to the foregoing sequences under stringent conditions.

The present invention also includes an isolated voltage gated Na^+ channel that is preferentially expressed in dorsal root ganglia or trigeminal ganglia. In a preferred embodiment, the channel has the amino acid sequence of Figure 2 (SEQ ID NO: 3), Figure 7B (SEQ ID NO: 5), Figure 8B (SEQ ID NO: 8) or Figure 11B (SEQ ID NO: 42), or is encoded by a nucleic acid having the sequence shown in Figure 2 (SEQ ID NO: 3), Figure 7B (SEQ ID

NO: 5), Figure 8B (SEQ ID NO: 8) or Figure 11B (SEQ ID NO: 42), allelic variants of said sequences or nucleic acids that hybridize to the foregoing sequences under stringent conditions. Peptide fragments of the channel are also included.

Another aspect of the invention is a method to identify an agent that modulates the activity of the NaN channel, comprising the steps of bringing the agent into contact with a cell that expresses the Na^+ channel on its surface and measuring depolarization, or any resultant changes in the sodium current. The measuring step may be accomplished with voltage clamp measurements, by measuring depolarization, the level of intracellular sodium or by measuring sodium influx.

Another aspect of the invention is a method to identify an agent that modulates the transcription or translation of mRNA encoding the NaN channel. The method comprises the steps of bringing the agent into contact with a cell that expresses the Na^+ channel on its surface and measuring the resultant level of expression of the Na^+ channel.

The invention also includes a method to treat pain, paraesthesia and hyperexcitability phenomena in an animal or human subject by administering an effective amount of an agent capable of modulating, such as by inhibiting or enhancing, Na^+ current flow through NaN channels in DRG or trigeminal neurons. The method may include administering an effective amount of an agent capable of modulating the transcription or translation of mRNA encoding the NaN channel.

Another aspect of the invention is an isolated nucleic acid that is antisense to the nucleic acids described above. In a preferred embodiment, the antisense nucleic acids are of sufficient length to modulate the expression of NaN channel mRNA in a cell containing the mRNA.

Another aspect of the invention is a scintigraphic method to image the loci of pain generation or provide a measure the level of pain associated with DRG or trigeminal neuron mediated hyperexcitability in an animal or human subject by administering labeled monoclonal antibodies or other labeled ligands specific for the NaN Na^+ channel.

Another aspect of the invention is a method to identify tissues, cells and cell types that express the NaN sodium channel. This method comprises the step of detecting NaN on the cell surface, or en route to the cell surface, or the presence of NaN encoding mRNA.

The present invention further includes a method of producing a transformed cell that expresses an exogenous NaN encoding nucleic acid, comprising the step of transforming the cell with an expression vector comprising an isolated nucleic acid having the sequence shown in Figures 1, 7A, 8A or 11A, allelic variants of said sequences or nucleic acids that hybridize to the foregoing sequences under stringent conditions, together with appropriate regulatory and expression control elements. The invention also includes a method of producing recombinant NaN protein, comprising the step of culturing the transformed host under conditions in which the NaN sodium channel or protein is expressed, and recovering the NaN protein.

The invention also includes an isolated antibody specific for the *NaN* channel or polypeptide fragment thereof. The isolated antibody may be labeled.

Another aspect of the invention includes a therapeutic composition comprising an effective amount of an agent capable of decreasing rapidly repriming sodium current flow in axotomized, inflamed or otherwise injured DRG neurons or in normal DRG neurons that are being driven to fire at high frequency. The invention also includes a method to treat acute pain or acute or chronic neuropathic or inflammatory pain and hyperexcitability phenomena in an animal or a human patient by administering the therapeutic composition.

The present invention also includes a method to screen candidate compounds for use in treating pain and hyperexcitability phenomena by testing their ability to alter the expression or activity of an NaN channel mRNA or protein in axotomized, inflamed or otherwise injured DRG neurons.

BRIEF DESCRIPTION OF THE DRAWING FIGURES

Figure 1 shows the sequence of the rat *NaN* cDNA (SEQ ID NO: 3).

Figure 2 shows the putative amino acid sequence of the rat *NaN* cDNA (SEQ ID NO: 3). Predicted transmembrance segments of domains I - IV are underlined. The amino acid serine "S" in DI-SS2, implicated in the TTX-R phenotype, is in bold face type.

Figure 3 presents a schematic diagram of predicted secondary structure of the *NaN* a-subunit.

Figure 4 shows the results of RT-PCR analysis for α-*NaN* in extracts of various tissues using *NaN*-specific primers. *NaN* is abundantly expressed in dorsal root and trigeminal ganglia. Low levels of *NaN* are detected in cerebral hemisphere and retina tissues. No detectable *NaN* signal is seen in cerebellum, optic nerve, spinal cord, sciatic nerve, superior cervical ganglia, skeletal muscle, cardiac muscle, adrenal gland, uterus, liver and kidney.

Figure 5 shows the tissue distribution of α-*NaN* by *in situ* hybridization. Trigeminal ganglion neurons show moderate-to-high hybridization signal (A). Dorsal root ganglion neurons show moderate-to-high hybridization signal in small neurons (B). Hybridization signal is attenuated in large neurons (arrow). (C) Sense probe shows no signal in DRG neurons. No hybridization signal is seen in spinal cord, cerebellum and liver (D-F) . All tissues are from adult Sprague-Dawley rat (scale bars = 50 micrometer).

Figure 6 shows the predicted lengths of domain I amplification products of rat α-subunits and their subunit-specific restriction enzyme profile.

Figure 7 sets forth the (A) nucleotide (SEQ ID NO: 4) and (B) amino acid (SEQ ID NO: 5) sequences of the murine NaN.

Figure 8 is a partial (A) nucleotide sequence (SEQ ID NO: 6) of the human *NaN* and partial (B) amino acid sequence (SEQ ID NO: 8) of the human NaN protein.

Figure 9 shows cultures of DRG neurons obtained from L4/5 ganglia of adult rats that were reacted with antibody to NaN and then processed for immunofluorescent localization. (A-B) NaN immunostaining is prominent within the cell bodies of DRG neurons. (C) NaN is

present in the neuritic outgrowths, as well as the cell bodies, of DRG neurons. Nomarski (D) and fluorescent (D') images of a neuron that does not express NaN protein.

Figure 10 shows the location of Scn1 la and related genes on distal mouse chromosome 9. (A) Haplotypes from the Jackson BSS backcross. Black boxes represent C57BL/6J alleles and white boxes represent SPRET/Ei alleles. The number of animals with each haplotype is given below each column. Missing data was inferred from adjacent data when typing was ambiguous. (B) Map of distal chromosome 9 based on data in (A). Positions of *Scn5a* and *Scn10a* from the MGD consensus map and the locations of the human orthologs are indicated. Numbers are cM positions on the consensus map (http://www.informatics.jax.org/bin/ccr/index).

Figure 11 shows the (A) cDNA nucleotide sequence (SEQ ID NO: 41) of the human *NaN* gene spanning the complete open reading frame and (B) sets forth the amino acid sequence (SEQ ID NO: 42) of the full length human NaN protein.

DETAILED DESCRIPTION

The present invention relates to a novel gene that Applicants have discovered, called *NaN*. *NaN* encodes a previously unidentified protein, referred to herein as NaN, that belongs to the a-subunit voltage-gated sodium channel protein family and that produces a TTX-R sodium current. Such channels underlie the generation and propagation of impulses in excitable cells like neurons and muscle fibers. *NaN* is a novel sodium channel, with a sequence distinct from other, previously identified, channels. The preferential expression of *NaN* on sensory, but not other neurons, makes it a very useful target for diagnostic and/or therapeutic uses in relation to acute and/or chronic pain pathologies..

A. Definitions

This specification uses several technical terms and phrases which are intended to have the following meanings:

The phrase "modulate" or "alter" refers to up- or down-regulating the level or activity of a particular receptor, ligand or current flow. For example an agent might modulate Na^+ current flow by inhibiting (decreasing) or enhancing (increasing) Na^+ current flow. Similarly,

an agent might modulate the level of expression of the NaN sodium channel or the activity of the NaN channels that are expressed.

The phrase "sodium current" or "Na^+ current" means the flow of sodium ions across a cell membrane, often through channels (specialized protein molecules) that are specifically permeable to certain ions, in this case sodium ions.

The phrase "voltage gated" means that the ion channel opens when the cell membrane is in a particular voltage range. Voltage-sensitive sodium channels open when the membrane is depolarized. They then permit Na^+ ions to flow into the cell, producing further depolarization. This permits the cell to generate electrical impulses (also known as "action potentials").

The phrase "rapidly repriming" means that the currents recover from inactivation more rapidly than do such currents in most other voltage gated sodium channel family members.

The terms "TTX-R" and "TTX-S" means that the flow of current through a cell membrane is, respectively, resistant or sensitive to tetrodotoxin (a neurotoxin produced in certain species) at a concentration of about 100 nM.

The phrase "peripheral nervous system (PNS)" means the part of the nervous system outside of the brain and spinal cord, *i.e.*, the spinal roots and associated ganglia such as dorsal root ganglia (DRG) and trigeminal ganglia, and the peripheral nerves.

The phrase "inhibits Na^+ current flow" means that an agent has decreased such current flow relative to a control cell not exposed to that agent. A preferred inhibitor will selectively inhibit such current flow, without affecting the current flow of other sodium channels; or it will inhibit Na^+ current in the channel of interest to a much larger extent than in other channels.

The phrase "enhances Na^+ current flow" means that an agent has increased such current flow relative to a control cell not exposed to that agent. A preferred agent will selectively increase such current flow, without affecting the current flow of other sodium channels; or it will increase Na^+ current in the channel of interest to a much larger extent than in other channels.

The phrase "specifically hybridizes" refers to nucleic acids which hybridize under highly stringent or moderately stringent conditions to the nucleic acids encoding the NaN sodium channel, such as the DNA sequence of SEQ ID NO: 1, 4, 6 or 41.

The phrase "isolated nucleic acid" refers to nucleic acids that have been separated from or substantially purified relative to contaminant nucleic acids encoding other polypeptides. "Nucleic acids" refers to all forms of DNA and RNA, including cDNA molecules and antisense RNA molecules.

The phrase "RT-PCR" refers to the process of reverse transcription of RNA (RT) using the enzyme reverse transcriptase, followed by the amplification of certain cDNA templates using the polymerase chain reaction (PCR); PCR requires generic or gene-specific primers and thermostable DNA polymerase, for example, *Taq* DNA polymerase.

The phrase "preferentially expressed" means that voltage gated Na^+ channel is expressed in the defined tissues in detectably greater quantities than in other tissues. For instance, a voltage gated Na^+ channel that is preferentially expressed in dorsal root ganglia or trigeminal ganglia is found in detectably greater quantities in dorsal root ganglia or trigeminal ganglia when compared to other tissues or cell types. The quantity of the voltage gated Na^+ channel may be detected by any available means, including the detection of specific RNA levels and detection of the channel protein with specific antibodies.

B. Characterization of the NaN Sodium Channel

The present invention relates to a previously unidentified, voltage-gated sodium channel a-subunit (*NaN*), predicted to be TTX-R, voltage-gated, and preferentially expressed in sensory neurons innervating the body (dorsal root ganglia or DRG) and the face (trigeminal ganglia). The predicted open reading frame (ORF), the part of the sequence coding for the NaN protein molecule, has been determined with the putative amino acid sequence from different species (rat, mouse, human) presented in Figures 2 (SEQ ID NO: 3), 7B (SEQ ID NO: 5), 8B (SEQ ID NO: 8) or 11B (SEQ ID NO: 42).

All of the relevant landmark sequences of voltage-gated sodium channels are present in NaN at the predicted positions, indicating that *NaN* belongs to the sodium channel family.

But NaN is distinct from all other previously identified Na channels, sharing a sequence identity of less than 53% with each one of them. *NaN* is distinct from *SNS*, the only other TTX-R Na^+ channel subunit that has been identified, until our discovery, in PNS. We have identified and cloned *NaN* without using any primers or probes that are based upon or specific to *SNS*. Moreover, *NaN* and *SNS* share only 47% similarity of their predicted open reading frame (ORF), comparable to the limited similarity of *NaN* to all subfamily 1 members.

The low sequence similarity to existing a-subunits clearly identifies *NaN* as a novel gene, not simply a variant of an existing channel. Sequence variations compared to the other voltage-gated channels indicate that NaN may be the prototype of a novel and previously unidentified, third class of TTX-R channels that may possess distinct properties compared to *SNS*. *NaN* and *SNS*, which are present in nociceptive DRG and trigeminal neurons, may respond to pharmacological interventions in different ways. The preferential expression of NaN in sensory DRG and trigeminal neurons provides a target for selectively modifying the behavior of these nerve cells while not affecting other nerve cells in the brain and spinal cord. A further elucidation of the properties of NaN channels will be important to understand more fully the effects of drugs designed to modulate the function of the "TTX-R" currents which are characteristic of DRG nociceptive neurons and which contribute to the transmission of pain messages, and to abnormal firing patterns after nerve injury and in other painful conditions..

C. NaN Nucleic Acids

Nucleic acid molecules of the invention include the nucleotide sequences set forth in Figures 1, 7A, 8A and 11A as well as nucleotide sequences that encode the amino acid sequences of Figures 2, 7B, 8B and 11B. Nucleic acids of the claimed invention also include nucleic acids which specifically hybridize to nucleic acids comprising the nucleotide sequences set forth in Figures 1, 7A, 8A and 11A, or nucleotide sequences which encode the amino acid sequences of Figures 2, 7B, 8B and 11B. A nucleic acid which specifically hybridizes to a nucleic acid comprising that sequence remains stably bound to said nucleic acid under highly stringent or moderately stringent conditions. Stringent and moderately stringent conditions are those commonly defined and available, such as those defined by

Sambrook *et al.*, (1989) Molecular Cloning - A Laboratory Approach, Cold Spring Harbor Press or Ausubel *et al.*, (1995) Current Protocols in Molecular Biology, Greene Publishing. The precise level of stringency is not important, rather, conditions should be selected that provide a clear, detectable signal when specific hybridization has occurred.

Hybridization is a function of sequence identity (homology), G+C content of the sequence, buffer salt content, sequence length and duplex melt temperature (T[m]) among other variables (see Maniatis *et al.*, (1982) Molecular Cloning, Cold Spring Harbor Press). With similar sequence lengths, the buffer salt concentration and temperature provide useful variables for assessing sequence identity (homology) by hybridization techniques. For example, where there is at least 90 percent homology, hybridization is commonly carried out at 68°C in a buffer salt such as 6×SCC diluted from 20×SSC (see Sambrook *et al.*, (1989) Molecular Cloning - A Laboratory Approach, Cold Spring Harbor Press). The buffer salt utilized for final Southern blot washes can be used at a low concentration, *e.g.*, 0.1×SSC and at a relatively high temperature, *e.g.*, 68°C, and two sequences will form a hybrid duplex (hybridize). Use of the above hybridization and washing conditions together are defined as conditions of high stringency or highly stringent conditions. Moderately stringent conditions can be utilized for hybridization where two sequences share at least about 80 percent homology. Here, hybridization is carried out using 6×SSC at a temperature of about 50-55°C. A final wash salt concentration of about 1-3×SSC and at a temperature of about 60-68°C are used. These hybridization and washing conditions define moderately stringent conditions.

In particular, specific hybridization occurs under conditions in which a high degree of complementarity exists between two nucleic acid molecules. With specific hybridization, complementarity will generally be at least about 70%, 75%, 80%, 85%, preferably about 90-100%, or most preferably about 95-100%. When referring the human NaN sequence of SEQ ID NO:41 and 42, preferred homologous sequences will typically encode an NaN protein exhibiting at least about 81% amino acid sequence similarity or at least about 75% or 76% sequence identity to SEQ ID NO: 42. A more preferred human NaN sequence will contain a positively changed residue at amino acid 670, preferably an arginine residue.

As used herein, homology or identity is determined by **BLAST** (Basic Local Alignment Search Tool) analysis using the algorithm employed by the programs **blastp, blastn, blastx, tblastn** and **tblastx** (Karlin *et al.*, (1990) Proc. Natl. Acad. Sci. USA 87, 2264-2268 and Altschul, (1993) J. Mol. Evol. 36, 290-300, both of which are herein incorporated by reference) which are tailored for sequence similarity searching. The approach used by the **BLAST** program is to first consider similar segments between a query sequence and a database sequence, then to evaluate the statistical significance of all matches that are identified and finally to summarize only those matches which satisfy a preselected threshold of significance. For a discussion of basic issues in similarity searching of sequence databases (see Altschul *et al.*, Nat. Genet. (1994) 6, 119-129) which is herein incorporated by reference. The search parameters for **histogram, descriptions, alignments, expect** (*i.e.*, the statistical significance threshold for reporting matches against database sequences), **cutoff, matrix** and **filter** are at the default settings. The default scoring matrix used by **blastp, blastx, tblastn,** and **tblastx** is the **BLOSUM62** matrix (Henikoff *et al.*, (1992) Proc. Natl. Acad. Sci. USA 89, 10915-10919, herein incorporated by reference). For **blastn**, the scoring matrix is set by the ratios of **M** (*i.e.*, the reward score for a pair of matching residues) to **N** (i.e., the penalty score for mismatching residues), wherein the default values for **M** and **N** are 5 and -4, respectively.

The nucleic acids of the present invention can be used in a variety of ways in accordance with the present invention. For example, they can be used as nucleic acid probes to screen other cDNA and genomic DNA libraries so as to select by hybridization other DNA sequences that encode homologous *NaN* sequences. Contemplated nucleic acid probes could be RNA or DNA labeled with radioactive nucleotides or by non-radioactive methods (for example, biotin). Screening may be done at various stringencies (through manipulation of the hybridization Tm, usually using a combination of ionic strength, temperature and/or presence of formamide) to isolate close or distantly related homologs. The nucleic acids may also be used to generate primers to amplify cDNA or genomic DNA using polymerase chain reaction (PCR) techniques. The nucleic acid sequences of the present invention can also be used to identify adjacent sequences in the genome, for example, flanking sequences and regulatory elements of *NaN*. The nucleic acids may also be used to generate antisense primers or

constructs that could be used to modulate the level of gene expression of NaN. The amino acid sequence may be used to design and produce antibodies specific to NaN that could be used to localize NaN to specific cells and to modulate the function of NaN channels expressed on the surface of cells.

Nucleic acids of the invention also include recombinantly prepared altered NaN sequences. For instance, fusion proteins may be prepared with the open reading frames herein disclosed, or functional fragments thereof, and any available fusion protein. Nucleic acid molecules may also be prepared that encode chimeric NaN proteins, for instance, chimeric proteins comprising individual domains from different species. Such chimeric proteins include, but are not limited to, human NaN chimeras containing mouse or rat domains, or mouse or rat chimeras containing human domains. Preferred chimeras include human NaN with a rat or mouse domain surrounding the residue equivalent to amino acid 670 of human NaN.

D. Vectors and Transformed Host Cells

The present invention also comprises recombinant vectors containing and capable of replicating and directing the expression of nucleic acids encoding a NaN sodium channel in a compatible host cell. For example, the insertion of a DNA in accordance with the present invention into a vector using enzymes such as T4 DNA ligase, may be performed by any conventional means. Such an insertion is easily accomplished when both the DNA and the desired vector have been cut with the same restriction enzyme or enzymes, since complementary DNA termini are thereby produced. If this cannot be accomplished, it may be necessary to modify the cut ends that are produced by digesting back single-stranded DNA to produce blunt ends, or by achieving the same result by filling in the single-stranded termini with an appropriate DNA polymerase. In this way, blunt-end ligation may be carried out. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini. Such linkers may comprise specific oligonucleotide sequences that encode restriction site recognition sequences.

Any available vectors and the appropriate compatible host cells may be used (Sambrook *et al.*, (1989) Molecular Cloning - A Laboratory Approach, Cold Spring Harbor Press; Ausubel *et al.*, (1995) Current Protocols in Molecular Biology, Greene Publishing). Commercially available vectors, for instance, those available from New England Biolabs, Promega, Stratagene or other commercial sources are included.

The transformation of appropriate cell hosts with an rDNA (recombinant DNA) molecule of the present invention is accomplished by well known methods that typically depend on the type of vector used and host system employed. Frog oocytes can be injected with RNA and will express channels, but in general, expression in a mammalian cell line (such as HEK293 or CHO cells) is preferred. With regard to transformation of prokaryotic host cells, electroporation and salt treatment methods are typically employed (see, for example, Cohen *et al.*, (1972) Proc. Natl. Acad. Sci. USA 69, 2110-2114; and Maniatis *et al.*, (1982) Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Press). With regard to transformation of vertebrate cells with vectors containing rDNAs, electroporation, cationic lipid or salt treatment methods are typically employed (Graham *et al.*, (1973) Virology 52, 456-467; Wigler *et al.*, Proc. Natl. Acad. Sci. USA (1979) 76, 1373-1376).

Successfully transformed cells, *i.e.*, cells that contain an rDNA molecule of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an rDNA of the present invention can be cloned to produce single colonies. Cells from those colonies can be harvested, lysed and their DNA content examined for the presence of the rDNA using conventional methods (Southern, (1975) J. Mol. Biol. 98, 503-517) or the proteins produced from the cell assayed via an immunological method. If tags such as green fluorescent protein are employed in the construction of the recombinant DNA, the transfected cells may also be detected *in vivo* by the fluorescence of such molecules by cell sorting.

For transient expression of recombinant channels, transformed host cells for the measurement of Na^+ current or intracellular Na^+ levels are typically prepared by co-transfecting constructs into cells such as HEK293 cells with a fluorescent reporter plasmid (such as pGreen Lantern-1, Life Technologies) using the calcium-phosphate precipitation

technique (Ukomadu *et al.*, (1992) Neuron 8, 663-676). HEK293 cells are typically grown in high glucose DMEM (Life Technologies) supplemented with 10% fetal calf serum (Life Technologies). After forty-eight hours, cells with green fluorescence are selected for recording (Dib-Hajj *et al.*, (1997) FEBS Lett. 416, 11-14).

For preparation of cell lines continuously expressing recombinant channels, the *NaN* construct is cloned into other vectors that carry a selectable marker in mammalian cells. Transfections are carried out using the calcium phosphate precipitation technique (Ukomadu *et al.*, (1992) Neuron 8, 663-676). Human embryonic kidney (HEK-293), chinese hamster ovary (CHO) cells, derivatives of either or other suitable cell lines are grown under standard tissue culture conditions in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The calcium phosphate-DNA mixture is added to the cell culture medium and left for 15-20 hours, after which time the cells are washed with fresh medium. After 48 hours, antibiotic (G418) is added to select for cells which have acquired neomycin resistance. After 2-3 weeks in G418, 10-20 isolated cell colonies are harvested using sterile 10 ml pipette tips. Colonies are grown for another 4-7 days, split and subsequently tested for channel expression using whole-cell patch-clamp recording techniques and RT-PCR.

E. Method of Measuring Na^+ Current Flow

Na^+ currents are measured using patch clamp methods (Hamill *et al.*, (1981) Pflügers Arch. 391, 85-100), as described by Rizzo *et al.*, (1994) J. Neurophysiol. 72, 2796-2815 and Dib-Hajj *et al.*, (1997) FEBS Lett. 416, 11-14. For these recordings data are acquired on a MacIntosh Quadra 950 or similar computer, using a program such as Pulse (v 7.52, HEKA, German). Fire polished electrodes typically (0.8-1.5 MW) are fabricated from capillary glass using a Sutter P-87 puller or a similar instrument. In the most rigorous analyses, cells are usually only considered for analysis if initial seal resistance is <5 Gohm, they have high leakage currents (holding current <0.1 nA at -80 mV), membrane blebs, and an access resistance <5 Mohm. Access resistance is usually monitored throughout the experiment and data are not used if resistance changes occur. Voltage errors are minimized using series resistance compensation and the capacitance artifact is canceled using computer controlled

amplifier circuitry or other similar methods. For comparisons of the voltage dependence of activation and inactivation, cells with a maximum voltage error of ±10mV after compensation are used. Linear leak subtraction is usually used for voltage clamp recordings. Membrane currents are typically filtered at 5 KHz and sampled at 20 KHz. The pipette solution contains a standard solution such as: 140 mM CsF, 2 mM MgCl, 1 mM EGTA, and 10 mM Na-HEPES (pH 7.3). The standard bathing solution is usually 140 nM NaCl, 3 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES, and 10 mM glucose (pH 7.3).

Voltage clamp studies on transformed cells or DRG neurons, using methods such as intracellular patch-clamp recordings, can provide a quantitative measure of the sodium current density (and thus the number of sodium channels in a cell), and channel physiological properties. These techniques, which measure the currents that flow through ion channels such as sodium channels, are described in Rizzo *et al.*, (1995) Neurobiol. Dis. 2, 87-96. Alternatively, the blockage or enhancement of sodium channel function can be measured using optical imaging with sodium-sensitive dyes or with isotopically labeled Na. These methods which are described in Rose *et al.*, (1997) J. Neurophysiol. 78, 3249-3258 and by (Kimelberg & Waltz, (1988) The Neuronal Microenvironment (Boulton *et al.*, editors) Humana Press), measure the increase in intracellular concentration of sodium ions that occurs when sodium channels are open.

F. Measurement of Intracellular Sodium $[Na^+]_i$

The effects of various agents on cells that express Na^+ can be determined using ratiometric imaging of $[Na^+]_i$ using SBFI or other similar ion-sensitive dyes. In this method, as described by Sontheimer *et al.*, (1994) J. Neurosci. 14, 2464-2475, cytosolic-free Na^+ is measured using an indicator for Na^+, such as SBFI (sodium-binding benzofuran isophthalate (Harootunian *et al.*, (1989) J. Biol. Chem. 264, 19458-19467)); or a similar dye. Cells are first loaded with the membrane-permeable acetoxymethyl ester form of the dye (which is dissolved in dimethyl sulfoxide (DMSO) at a stock concentration of 10 mM). Recordings are obtained on the stage of a microscope using a ratiometric imaging setup (*e.g.*, from Georgia Instruments). Excitation light is provided at appropriate wavelengths (*e.g.*, 340:385 nm). Excitation light is

passed to the cells through a dichroic reflector (400 nm) and emitted light above 450 nm is collected. Fluorescence signals are amplified, *e.g.*, by an image intensifier (GenIISyS) and collected with a CCD camera, or similar device, interfaced to a frame grabber. To account for fluorescence rundown, the fluorescence ratio 340:385 is used to assay cytosolic-free Na^+.

For calibration of SBFI's fluorescence, cells are perfused with calibration solutions containing known Na^+ concentrations (typically 0 and 30 mM, or 0, 30, and 50 mM [Na^+]), and with ionophones such as gramicidin and monensin (see above) after each experiment. As reported by Rose & Ransom, (1996) J. Physiol. (Lond) 491, 291-305, the 345/390 nm fluorescence ratio of intracellular SBFI changes monotonically with changes in $[Na^+]_i$. Experiments are typically repeated on multiple (typically at least four) different coverslips, providing statistically significant measurements of intracellular sodium in control cells, and in cells exposed to various concentrations of agents that may block, inhibit or enhance Na^+.

G. Method to Measure Na^+ Influx via Measuring ^{22}Na or ^{86}Rb

^{22}Na is a gamma emitter and can be used to measure Na^+ flux (Kimelberg & Waltz, (1988) The Neuronal Microenvironment (Boulton *et al.*, editors) Humana Press), and $^{86}Rb^+$ can be used to measure Na^+/K^+-ATPase activity (Sontheimer *et al.*, (1994) J. Neurosci. 14, 2464-2475). $^{86}Rb^+$ ions are taken up by the Na^+/K^+-ATPase-like K^+ ions, but have the advantage of a much longer half-life than $^{42}K^+$ (Kimelberg & Mayhew (1975) J. Biol. Chem. 250, 100-104). Thus, measurement of the unidirectional ouabain-sensitive $^{86}Rb^+$ uptake provides a quantitative method for assaying Na^+/K^+-ATPase activity which provides another indicator of the electrical firing of nerve cells. Following incubation of cells expressing *NaN* with the isotope $^{22}Na^+$, the cellular content of the isotope is measured by liquid scintillation counting or a similar method, and cell protein is determined using a method such as the bicinchoninic acid protein assay (Smith *et al.*, (1985) Anal. Biochem. 150, 76-85) following the modifications described by Goldschmidt & Kimelberg (1989) Anal. Biochem. 177, 41-45 for cultured cells. ^{22}Na and $^{86}Rb^+$ fluxes are determined in the presence and absence of agents that may block, inhibit, or enhance NaN. This permits determination of the actions of these agents on NaN.

H. Method to Identify Agents that Modulate NaN-Mediated Current

Several approaches can be used to identify agents that are able to modulate (*i.e.*, block or augment) the Na^+ current through the NaN sodium channel. In general, to identify such agents, a model cultured cell line that expresses the NaN sodium channel is utilized, and one or more conventional assays are used to measure Na^+ current. Such conventional assays include, for example, patch clamp methods, the ratiometric imaging of $[Na^+]_i$ and the use of ^{22}Na and ^{86}Rb as described above.

In one embodiment of the present invention, to evaluate the activity of a candidate compound to modulate Na^+ current, an agent is brought into contact with a suitable transformed host cell that expresses *NaN*. After mixing or appropriate incubation time, the Na^+ current is measured to determine if the agent inhibited or enhanced Na^+ current flow.

Agents that inhibit or enhance Na^+ current are thereby identified. A skilled artisan can readily employ a variety of art-recognized techniques for determining whether a particular agent modulates the Na^+ current flow.

Because Na^+ is preferentially expressed in pain-signaling cells, one can also design agents that block, inhibit, or enhance Na^+ channel function by measuring the response of laboratory animals, treated with these agents, to acute, inflammatory or chronic pain. In one embodiment of this aspect of the invention, laboratory animals such as rats are treated with an agent for instance, an agent that blocks or inhibits (or is thought to block or inhibit) NaN. The response to various painful stimuli are then measured using tests such as the tail-flick test and limb withdrawal reflex, and are compared to untreated controls. These methods are described by Dubner, (1994) Textbook of Pain (Wall & Melzack, editors) Churchill Livingstone Publishers. In another embodiment of this aspect of the invention, laboratory animals such as rats are subjected to localized injection of pain-producing inflammatory agents such as formalin (Dubuisson & Dennis (1977) Pain 4, 161-74), Freunds adjuvant (Iadarola *et al.*, (1988) Pain 35, 313-326) or carageenan, or are subjected to nerve constriction (Bennett & Xie, (1988) Pain 33, 87-107; Kim & Chung (1992) Pain 50, 355-363) or nerve transection (Seltzer *et al.*, (1990) Pain 43, 205-218) which produce persistent pain. The response to various normal and painful stimuli are then measured, for example, by measuring the latency to

withdrawal from a warm or hot stimulus (Dubner, (1994) Textbook of Pain (Wall & Melzack, editors) Churchill Livingstone Publishers) so as to compare control animals and animals treated with agents that are thought to modify NaN.

The preferred inhibitors and enhancers of NaN preferably will be selective for the NaN Na^+ channel. They may be totally specific (like tetrodotoxin, TTX, which inhibits sodium channels but does not bind to or directly effect any other channels or receptors), or relatively specific (such as lidocaine which binds to and blocks several types of ion channels, but has a predilection for sodium channels). Total specificity is not required for an inhibitor or enhancer to be efficacious. The ratio of its effect on sodium channels vs. other channels and receptors, may often determine its effect and effects on several channels, in addition to the targeted one, may be efficacious (Stys *et al.*, (1992) J. Neurophysiol. 67, 236-240). Modulators of NaN may be combined with or coadministered with agents that modulate other channels expressed in primary sensory neurons, including but not limited to PN1/hNE and SNS/PN3 (Waxman (1999) Pain Supplement 6:S133-140).

It is contemplated that modulating agents of the present invention can be, as examples, peptides, small molecules, naturally occurring and other toxins and vitamin derivatives, as well as carbohydrates. A skilled artisan can readily recognize that there is no limit as to the structural nature of the modulating agents of the present invention. Screening of libraries of molecules may reveal agents that modulate NaN or current flow through it. Similarly, naturally occurring toxins (such as those produced by certain fish, amphibians and invertebrates) can be screened. Such agents can be routinely identified by exposing a transformed host cell or other cell which expresses a sodium channel to these agents and measuring any resultant changes in Na^+ current.

I. Recombinant Protein Expression, Synthesis and Purification

Recombinant NaN proteins can be expressed, for example, in *E. coli* strains HB101, DH5a or the protease deficient strain such as CAG-456 and purified by conventional techniques.

The peptide agents of the invention can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if non-gene-encoded amino acids are to be included.

J. Antibodies and Immunodetection

Another class of agents of the present invention are antibodies immunoreactive with the Na^+ channel. These antibodies may block, inhibit, or enhance the Na^+ current flow through the channel. Antibodies can be obtained by immunization of suitable mammalian subjects with peptides, containing as antigenic regions, those portions of NaN, particularly (but not necessarily) those that are exposed extracellularly on the cell surface. Such immunological agents also can be used in competitive binding studies to identify second generation inhibitory agents. The antibodies may also be useful in imaging studies, once appropriately labeled by conventional techniques.

K. Production of Transgenic Animals

Transgenic animals containing and mutant, knock-out or modified *NaN* genes are also included in the invention. Transgenic animals wherein both *NaN* and the SNS/PN3 gene are modified, disrupted or in some form modified are also included in the present invention. Transgenic animals are genetically modified animals into which recombinant, exogenous or cloned genetic material has been experimentally transferred. Such genetic material is often referred to as a "transgene". The nucleic acid sequence of the transgene, in this case a form of *NaN*, may be integrated either at a locus of a genome where that particular nucleic acid sequence is not otherwise normally found or at the normal locus for the transgene. The transgene may consist of nucleic acid sequences derived from the genome of the same species or of a different species than the species of the target animal.

The term "germ cell line transgenic animal" refers to a transgenic animal in which the genetic alteration or genetic information was introduced into a germ line cell, thereby

conferring the ability of the transgenic animal to transfer the genetic information to offspring. If such offspring in fact possess some or all of that alteration or genetic information, then they too are transgenic animals.

The alteration or genetic information may be foreign to the species of animal to which the recipient belongs, foreign only to the particular individual recipient, or may be genetic information already possessed by the recipient. In the last case, the altered or introduced gene may be expressed differently than the native gene.

Transgenic animals can be produced by a variety of different methods including transfection, electroporation, microinjection, gene targeting in embryonic stem cells and recombinant viral and retroviral infection (see, *e.g.*, U.S. Patent No. 4,736,866; U.S. Patent No. 5,602,307; Mullins *et al.*, (1993) Hypertension 22, 630-633; Brenin *et al.*, (1997) Surg. Oncol. 6, 99-110; Tuan (1997) Recombinant Gene Expression Protocols, Humana Press).

A number of recombinant or transgenic mice have been produced, including those which express an activated oncogene sequence (U.S. Patent No. 4,736,866); express simian SV 40 T-antigen (U.S. Patent No. 5,728,915); lack the expression of interferon regulatory factor 1 (IRF-1) (U.S. Patent No. 5,731,490); exhibit dopaminergic dysfunction (U.S. Patent No. 5,723,719); express at least one human gene which participates in blood pressure control (U.S. Patent No. 5,731,489); display greater similarity to the conditions existing in naturally occurring Alzheimer's disease (U.S. Patent No. 5,720,936); have a reduced capacity to mediate cellular adhesion (U.S. Patent No. 5,602,307); possess a bovine growth hormone gene (Clutter *et al.*, (1996) Genetics 143, 1753-1760); or, are capable of generating a fully human antibody response (McCarthy, (1997) Lancet 349, 405).

While mice and rats remain the animals of choice for most transgenic experimentation, in some instances it is preferable or even necessary to use alternative animal species. Transgenic procedures have been successfully utilized in a variety of non-murine animals, including sheep, goats, pigs, dogs, cats, monkeys, chimpanzees, hamsters, rabbits, cows and guinea pigs (see Kim *et al.*, (1997) Mol. Reprod. Dev. 46, 515-526; Houdebine, (1995) Reprod. Nutr. Dev. 35, 609-617; Petters (1994) Reprod. Fertil. Dev. 6, 643-645; Schnieke *et al.*, (1997) Science 278, 2130-2133; Amoah, (1997) J. Animal Sci. 75, 578-585).

The method of introduction of nucleic acid fragments into recombination competent mammalian cells can be by any method which favors co-transformation of multiple nucleic acid molecules. Detailed procedures for producing transgenic animals are readily available to one skilled in the art, including the disclosures in U.S. Patent No. 5,489,743 and U.S. Patent No. 5,602,307.

The specific examples presented below are illustrative only and are not intended to limit the scope of the invention.

EXAMPLES

Example 1: Cloning and Characterization of the Rat *NaN* Coding Sequence

a. *RNA Preparation*

Dorsal root ganglia (DRG) from the lumber region (L4-L5) were dissected from adult Sprague-Dawley rats and total cellular RNA was isolated by the single step guanidinum isothiocyanate-acid phenol procedure (Chomczynski, (1987) Anal. Biochem. 162, 156-159). For analytical applications, DRG tissues were dissected from a few animals at a time. The quality and relative yield of the RNA was assessed by electrophoresis in a 1% agarose gel. Because of the limited starting material (four DRGs weigh on average 10 mg), quantifying the RNA yield was not attempted. PolyA+ RNA was purified from about 300 mg of total DRG RNA (28 animals) using the PolyATract isolation system according to the manufacturers recommendations (Promega). Half of the purified RNA was used for the preparation of Marathon cDNA (see below) without further quantification.

b. *Reverse Transcription*

For analytical applications, first strand cDNA was synthesized essentially as previously described (Dib-Hajj *et al.*, (1996) FEBS Lett. 384, 78-82). Briefly, total RNA was reverse transcribed in a 25 ml final volume using 1mM random hexamer (Boehringer Mannheim) and 500 units SuperScript II reverse transcriptase (Life Technologies) in the presence of 100 units of RNase Inhibitor (Boehringer Mannheim). The reaction buffer

consisted of 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT and 125 mM dNTP. The reaction was allowed to proceed at 37°C for 90 minutes, 42°C for 30 minutes, then terminated by heating to 65°C for 10 minutes.

c. *First-Strand cDNA Synthesis*

The Marathon cDNA synthesis protocol was followed according to the manufacturer's instruction as summarized below (all buffers and enzymes are purchased from the manufacturer (Clontech):

Combine the following reagents in a sterile 0.5-ml microcentrifuge tube: 1 mg (1-4 ml) PolyA$^+$ RNA sample, one ml cDNA Synthesis Primer (10 mM) and sterile water to a final volume of 5 ml. Mix contents and spin the tube briefly in a microcentrifuge. Incubate the mixture at 70°C for two minutes, then immediately quench the tube on ice for two minutes Touch-spin the tube briefly to collect the condensation. Add the following to each reaction tube: 2 ml 5× First-Strand Buffer, 1 ml dNTP Mix (10 mM), 1 ml [α-^{32}P]dCTP (1 mCi/ml), 1 ml AMV Reverse Transcriptase (20 units/ml) for a 10 ml volume. The radiolabeled dCTP is optional (used to determine yield of cDNA) and is replaced by sterile H_2O if not used. Mix the contents of the tube by gently pipetting and touch-spin the tube to collect the contents at the bottom. Incubate the mixture at 42°C for one hour in an air incubator to reduce condensation and enhance the yield of the first strand cDNA. Place the tube on ice to terminate first-strand synthesis.

d. *Second-Strand cDNA Synthesis*

Combine the following components in the reaction tube from above: 48.4 ml Sterile water, 16 ml 5× Second-Strand Buffer, 1.6 ml dNTP Mix (10 mM), 4 ml 20× Second-Strand Enzyme Cocktail for an 80 ml total volume. Mix the contents thoroughly with gentle pipetting and spin the tube briefly in a microcentrifuge. Incubate the mixture at 16°C for 1.5 hours then add 2 ml (10 units) of T4 DNA Polymerase, mix thoroughly with gentle pipetting and incubate the mixture at 16°C for 45 minutes. Add 4 ml of the EDTA/Glycogen mix to terminate second-strand synthesis. Extract the mixture with an equal volume of buffer-

saturated (pH 7.5) phenol:chloroform:isoamyl alcohol (25:24:1). Mix the contents thoroughly by vortexing and spin the tube in a microcentrifuge at maximum speed (up to 14,000 rpm or 13000×g), 4°C for ten minutes to separate layers. Carefully transfer the top aqueous layer to a clean 0.5-ml tube. Extract the aqueous layer with 100 ml of chloroform:isoamyl alcohol (24:1), vortex, and spin the tube as before to separate the layers. Collect the top layer into a clean 0.5-ml microcentrifuge tube. Ethanol precipitate the double-stranded cDNA by adding one-half volume of 4 M Ammonium Acetate and 2.5 volumes of room-temperature 95% ethanol. Mix thoroughly by vortexing and spin the tube immediately in a microcentrifuge at top speed, room temperature for twenty minutes Remove the supernatant carefully and wash the pellet with 300 ml of 80% ethanol. Spin the tube as before for 10 minutes and carefully remove the supernatant. Air dry the pellet for up to 10 minutes and dissolve the cDNA in 10 ml of sterile H_2O and store at -20°C. Analyze the yield and size of cDNA by running 2 ml of the cDNA solution on a 1.2% agarose/EtBr gel with suitable DNA size markers (for example, the 1 kilobp ladder, Gibco-BRL). If EtBr staining does not show a signal and [α-^{32}P]dCTP was included in the reaction, dry the agarose gel on a vacuum gel drying system and expose an x-ray film to the gel overnight at -70°C.

e. *Adaptor Ligation*

Combine these reagents in a 0.5-ml microcentrifuge test tube, at room temperature, and in the following order: 5 ml double-stranded cDNA, 2 ml Marathon cDNA Adaptor (10 mM), 2 ml 5× DNA Ligation Buffer, 1 ml T4 DNA Ligase (1 unit/ml) for a 10 ml final volume. Mix the contents thoroughly with gentle pipetting and spin the tube briefly in a microcentrifuge. Incubate at either: 16°C overnight; or room temperature (19-23°C) for three to four hours. Inactivate the ligase enzyme by heating the mixture at 70°C for five minutes. Dilute 1 ml of this reaction mixture with 250 ml of Tricine-EDTA buffer and use for RACE protocols. Store the undiluted adaptor-ligated cDNA at -20°C for future use.

f. *PCR*

For the initial discovery of *NaN*, we used generic primers designed against highly conserved sequences in domain 1 (D1) of a-subunits I, II and III and later added more primers to accommodate the new a-subunits that were discovered. Thus, generic primers were used that recognize conserved sequences in all known Na^+ channels. The middle of the amplified region shows significant sequence and length polymorphism (Figure 6) and (Gu *et al.*, (1997) J. Neurophysiol. 77, 236-246; Fjell *et al.*, (1997) Mol. Brain Res. 50, 197-204). Due to codon degeneracy, 4 forward primers (F1-F4) and 3 reverse primers (R1-R3) were designed to ensure efficient priming from all templates that might have been present in the cDNA pool (Table 1); however, any of these primers may bind to multiple templates depending on the stringency of the reaction. Forward primer F1 matches subunits aI, aIII; aNa6; aPN1; am1, arH1 and aSNS/PN3. Sequences of individual subunits show 1 or 2 mismatches to this primer: T to C at position 16 and A to G at position 18 (aNa6); C to R at position 6 (am1); A to G at position 18 (arH1) and T to C at position 3 (aSNS). Forward primer F2 matches subunit aII. Forward primer F3 perfectly matches aNa6 and also matches arH1 with a single mismatch of C to T at position 16. Reverse primer R1 matches subunits aI, aII, aIII, aNa6, aPN1, am1 and arH1. This primer has mismatches compared to 4 subunits: G to A at position 3, A to G at position 4 and T to G at position 7 (aI); T to C at position 1 and A to G at position 19 (aPN1); G to A at position 3 and A to G at position 7 (am1); an extra G after position 3, GC to CT at positions 14-15, and A to T at position 21 (arH1). Reverse primer R2 matches subunit aSNS/PN3.

Table 1: Generic and *NaN*-specific primers used for the identification and cloning of *NaN*. All primers except the marathon primers, were synthesized at the department of Pathology, Program for Critical Technologies in Molecular Medicine, Yale University.

Forward Primers	Reverse Primers
1. GACCCRTGGAATTGGTTGGA (SEQ ID NO: 9)	1. CAAGAAGGCCCAGCTGAAGGTGTC (SEQ ID NO: 15)
2. AATCCCTGGAATTGGTTGGA (SEQ ID NO: 10)	2. GAGGAATGCCCACGCAAAGGAATC (SEQ ID NO: 16)
3. GACCCGTGGAACTGGTTAGA (SEQ ID NO: 11)	3. AAGAAGGGACCAGCCAAAGTTGTC (SEQ ID NO: 17)
4. GATCTTTGGAACTGGCTTGA (SEQ ID NO: 12)	4. ACYTCCATRCANWCCCACAT (SEQ ID NO: 18)
5. AACATAGTGCTGGAGTTCAGG (SEQ ID NO: 13)	5. AGRAARTCNAGCCARCACCA (SEQ ID NO: 19)
6. GTGGCCTTTGGATTCCGGAGG (SEQ ID NO: 14)	6. TCTGCTGCCGAGCCAGGTA (SEQ ID NO: 20)
	7. CTGAGATAACTGAAATCGCC (SEQ ID NO: 21)
Marathon AP-1 CCATCCTAATACGACTCACTATAGGGC (SEQ ID NO: 22)	
Marathon AP-2 ACTCACTATAGGGCTCGAGCGGC (SEQ ID NO: 23)	

The respective mouse atypical sodium channel mNa$_v$2.3 sequence was used to design forward primer F4 and reverse primer R3 to amplify the analogous sequence from aNaG, the presumed rat homolog of mNa$_v$2.3 (Felipe *et al.*, (1994) J. Biol. Chem. 269, 30125-301231). The amplified sequence was cloned into the *Srf* I site of the vector pCR-Script (Stratagene). The nucleotide sequence of this fragment shows 88% identity to the respective sequence of mNa$_v$2.3 (Dib-Hajj & Waxman, unpublished). The restriction enzyme *Xba* I was found to be unique to this subunit. Recently, the sequence of a full length cDNA clone of putative sodium channel, NaG-like (SCL-11:Y09164), subunit was published (Akopian *et al.*, (1997) FEBS

Lett. 400, 183-187). The published sequence is 99% identical to our sequence and confirms the size and restriction enzyme polymorphism of the NaG PCR product.

The predicted lengths of amplified products and subunit-specific restriction enzyme recognition sites are shown in Figure 6. All subunit sequences are based on Genbank database (accession numbers: aI: X03638; αII: X03639; αIII: Y00766; αNa6: L39018; αhNE-Na: X82835; αm1 M26643; αrH1 M27902 and αSNS X92184; mNa 2.3 L36719). Subsequently, amplification of NaN sequences 3' terminal to the aforementioned fragment was achieved using NaN-specific primers and two generic reverse primers, R4 and R5. The sequence of the R4 primer was based on the amino acid sequence MWV/DCMEV (SEQ ID NO: 38) located just N-terminal to domain II S6 segment (see schematic diagram of Figure 3 of voltage-gated sodium channel a-subunits for reference). The sequence of the R5 primer is based on the amino acid sequence AWCWLDFL (SEQ ID NO: 43) which forms the N-terminal portion of domain III S3 segment.

Amplification was typically performed in 60 m1 volume using one m1 of the first strand cDNA, 0.8mM of each primer and 1.75 units of Expand Long Template DNA polymerase enzyme mixture (Boehringer Mannheim). Compared to conventional and thermostable DNA polymerases, Expand Long Template enzyme mixture increases the yield of the PCR products without an increase in non-specific amplification (Barnes, (1994) Proc. Natl. Acad. Sci. USA 91, 2216-2220; Cheng *et al.*, (1994) Proc. Natl. Acad. Sci. USA 91, 5695-5699). The PCR reaction buffer consisted of 50 mM Tris-HCl (pH 9.2), 16 mM $(NH_4)_2SO_4$, 2.25 mM $MgCl_2$, 2% (v/v) DMSO and 0.1% Tween 20. As described previously (Dib-Hajj *et al.*, (1996) FEBS Lett. 384, 78-82), amplification was carried out in two stages using a programmable thermal cycler (PTC-200, MJ Research). First, a denaturation step at 94°C for four minutes, an annealing step at 60°C for two minutes and an elongation step at 72°C for 90 seconds. Second, a denaturation step at 94°C for one minute, an annealing step at 60°C for one minute and an elongation step at 72°C for 90 seconds. The second stage was repeated 33 times for a total of 35 cycles, with the elongation step in the last cycle extended to ten minutes

Primary RACE amplification was performed in 50 ml final volume using 4 ml diluted DRG marathon cDNA template, 0.2 mM marathon AP-1 and NaN-specific primers, 3.5 units Expand Long Template enzyme mixture. Extension period was adjusted at 1 minute per 800 base pairs based on the expected product. 5' and 3' RACE amplification was performed using primer pairs marathon AP-1/*NaN*-specific R6 and *NaN*-specific F5/marathon AP-1, respectively. The PCR reaction buffer consisted of 50 mM Tris-HCl (pH 9.2), 16 mM $(NH_4)_2SO_4$, 3.0 mM $MgCl_2$, 2% (v/v) DMSO and 0.1% Tween 20. Amplification in three stages was performed in a programmable thermal cycler (PTC-200, MJ Research). An initial denaturation step at 92°C was carried out for two minutes This was followed by 35 cycles consisting of denaturation at 92°C for 20 seconds, annealing step at 60°C for one minute, and an elongation step at 68°C. Finally, an elongation step at 68°C was carried out for five minutes Nested amplification was performed using 2 ml of a 1/500 diluted primary RACE product in a final volume of 50 ml under similar conditions to the primary RACE reactions. Primer pairs AP-2/NaN-specific R7 and NaN-specific F6/marathon AP-2 were used for nested 5' and 3' RACE, respectively. Secondary RACE products were band isolated from 1% agarose gels and purified using Qiaex gel extraction kit (Qiagen).

A schematic diagram of the putative structure of *NaN* is shown in Figure 3. The length of the intracellular loops is highly variable both in sequence and length among the various subunits. The exception is the loop between domains III and IV.

Example 2: Determination of the Putative Rat Amino Acid Sequence for the NaN Channel

NaN-related clones and secondary RACE fragments were sequenced at the W. M. Keck Foundation Biotechnology Resource Lab, DNA sequencing group at Yale University. Sequence analysis including determination of the predicted amino acid sequence was performed using commercial softwares, Lasergene (DNAStar) and GCG. The putative amino acid sequence of NaN is shown in Figure 2. Predicted transmembrane segments of domains I - IV are underlined.

Example 3: Determination of the Murine NaN Sequence

Total RNA extraction from trigeminal ganglia of mice, purification of polyA+ RNA, and Marathon cDNA construction were done as previously described for the rat. The initial amplification was performed using rat *NaN* primers. The forward primer corresponds to nucleotides 765-787 of the rat sequence (5' CCCTGCTGCGCTCGGTGAAGAAG 3') (SEQ ID NO: 24), and the reverse primer corresponds to nucleotides 1156-1137 (negative strand) of the rat sequence (5' GACAAAGTAGATCCCAGAGG 3') (SEQ ID NO: 25). The amplification produced a fragment of the expected size. The sequence of this fragment demonstrated high similarity to rat *NaN*. Other fragments were amplified using different rat primers and primers designed based on the new mouse *NaN* sequence that was being produced. Finally, longer fragments were amplified using mouse Marathon cDNA template and mouse *NaN*-specific primers in combination with adaptor primers that were introduced during the Marathon cDNA synthesis. These fragments were sequenced using primer walking and assembled into Figure 7A.

Mouse *NaN* nucleotide sequence, like rat *NaN*, lacks the out-of-frame ATG at the -8 position relative to the translation initiation codon ATG at position 41 (Figure 7A). Translation termination codon TGA is at position 5314. A polyadenylation signal (AATAAA) is present at position 5789 and a putative 23 nucleotide polyA tail is present beginning at position 5800. The sequence encodes an ORF of 1765 amino acids (Figure 7B), which is 90% similar to rat NaN. The gene encoding NaN has been named Scn11a.

Chromosomal localization of mouse NaN

A genetic polymorphism between strains C57BL/6J and SPRET/Ei was identified by SSCP analysis of a 274 bp fragment from the 3'UTR of *Scn11a*. Genotyping of 94 animals from the BSS backcross panel (Rowe *et al.*, (1994) Mamm. Genome 5, 253-274) demonstrated linkage of *Scn11a* with markers on distal chromosome 9 (Figure 10). No recombinants were observed between *Scn11a* and the microsatellite marker *D9Mit19*. Comparison of our data with the MGD consensus map of mouse chromosome 9 revealed close linkage of *Scn11a* with the two other TTX-R voltage-gated sodium channels, *Scn5a* (George

et al., (1995) Cytogenet. Cell. Genet. 68, 67-70) and *Scn10a* (Kozak & Sangameswaran, (1996) Mamm. Genome 7, 787-788; Souslova *et al.*, (1997) Genomics 41, 201-209).

Example 4: Determination of a Partial and Complete Human NaN Coding Sequence

Human DRG tissue was obtained from a transplant donor. Total RNA extraction and cDNA synthesis were performed as described previously.

Forward primer corresponds to sequence 310-294 (minus strand) of EST AA446878. The sequence of the primer is 5' CTCAGTAGTTGGCATGC 3' (SEQ ID NO: 26). Reverse primer corresponds to sequence 270-247 (minus strand) of EST AA88521 1. The sequence of the primer is 5'GGAAAGAAGCACGACCACACAGTC 3' (SEQ ID NO: 27). Amplification was performed as previously described. PCR amplification was successful and a 2.1 kbp fragment was obtained. This fragment was gel purified and sent for sequencing by primer walking, similar to what is done for mouse *NaN*. The sequence of the ESTs is extended in both directions; the additional sequence shows highest similarity to rat and mouse *NaN*, compared to the rest of the subunits.

The sequence of a human 2.1 kbp fragment was obtained using the PCR forward and reverse primers for sequencing from both ends of the fragment. Two additional primers were used to cover the rest of the sequence. The sequence was then extended in the 5' direction using forward primer 1 (above) and human NaN reverse primer (5'-GTGCCGTAAACATGAGACTGTCG3') (SEQ ID NO: 44) near the 5' end of the 2.1 kbp fragment. The partial amino acid sequence is set forth in Figure 8B.

The partial ORF of the human *NaN* consists 1241 amino acids. The sequence is 64% identical to the corresponding sequence of rat *NaN* (73% similar, allowing for conservative substitutions) using the advanced BLAST program available at the National Center for Biotechnology Information. Using the Clustal method of alignment (Lasergene software, DNAStar) the human NaN is 68% and 69% similar to mouse and rat NaN, respectively. The respective mouse and rat sequences are 88% similar.

Further sequencing revealed the cDNA sequence spanning the full length open reading frame for the human *NaN* gene. This extended sequence is shown in Figure 11A (SEQ ID

NO: 41). In addition to the features noted with regard to the partial cDNA sequence (Figure 8A), notable features of the extended sequence include a translation start codon (ATG) at position 31 and a translation termination codon at position 5400. A recognizable polyadenylation signal has not been observed and presumably is located 3' of the disclosed sequence. The putative amino acid sequence of the human Nan protein is set forth in Figure 11B (SEQ ID NO: 42).

Example 5: Isolation of an Alternative Splicing Variant of Rat NaN

A rat NaN cDNA that encodes a C-terminal truncated version of the full-length rat NaN in Figures 1 and 2 was isolated by sequencing the insert of a rat cDNA clone. The variant NaN cDNA encodes an NaN protein lacking the 387 C-terminal amino acids of the full length NaN and containing a novel 94 amino acid stretch at the C-terminal end. The new sequence arises from the use of a cryptic donor splice site in exon 23 and a novel exon 23', which is located in intron 23. Thee novel C terminal amino acids are: AAGQAMRKQG DILGPNIHQF SQSSETPFLG CPQQRTCVSF VRPQRVLRVP WFPAWRTVTF LSRPRSSESS AWLGLVESSG WSGLPGESGP SSLL (SEQ ID NO: 28). The N-terminal amino acids of the truncated variant are identical to amino acids 1-1378 of the full length rat NaN of Figure 2. The alternative exon and the splicing pattern was confirmed by comparing the cDNA sequence and the genomic sequence in the respective region.

Example 6: Methods to Isolate Other *NaN* Sequences

a. *Isolation of NaN sequences from genomic DNA*

The genomic structure of three voltage-gated Na^+ channel a-subunits have already been determined (George *et al.*, (1993) Genomics 15, 598-606; Souslova *et al.*, (1997) Genomics 41, 201-209; McClatchey *et al.*, (1992) Hum. Mol. Genet. 1, 521-527; Wang *et al.*, (1996) Genomics 34, 9-16). These genes bear remarkable similarity in their organization and provide a predictable map of most of the exon/intron boundaries. Based on the available rat, mouse and human cDNA sequence of *NaN*, disclosed herein, PCR primers are designed to amplify *NaN* homologous sequences from other species using standard PCR protocols.

Alternatively, commercially available genomic DNA libraries are screened with *NaN*-specific probes (based on the rat, mouse, or more preferably, the human sequence) using standard library screening procedures (Sambrook *et al.*, (1989) Molecular Cloning - A Laboratory Approach, Cold Spring Harbor Press; Ausubel *et al.*, (1995) Current Protocols in Molecular Biology, Greene Publishing). This strategy yields genomic DNA isolates that can then be sequenced and the exon/intron boundaries determined by homology to the rat, mouse or human cDNA sequence.

b. *Isolation of full length NaN sequences allelic variants from autopsy or biopsy tissues*

For isolation of human ganglia total RNA, a full length *NaN* human cDNA homologue is isolated from human dorsal root ganglia or trigeminal ganglia or other cranial ganglia from post-mortem human material, foetuses or biopsy or surgical tissues. Total ribonucleic acid (RNA) is isolated from these tissues by extraction in guanidinium isothiocyanate (Saiki *et al.*, (1985) Science 230, 1350-1354) as described in Example 1.

For Determination of the full length transcript size of the human homologue of the rat NaN sodium channel cDNA, the method of determining transcript size is as described in Example 9.

Example 7: Production of human DRG cDNA library

A cDNA library from human DRG or trigeminal ganglia polyA+ RNA was prepared in Example 4 using standard molecular biology techniques (Sambrook *et al.*, (1989) Molecular Cloning - A Laboratory Approach, Cold Spring Harbor Press; Ausubel *et al.*, (1995) Current Protocols in Molecular Biology, Greene Publishing.

PolyA+ mRNA is hybridized to an oligo(dT) primer and the RNA is copied by reverse transcriptase into single strand cDNA. Then, the RNA in the RNA-DNA hybrid is fragmented by RNase H as *E. coli* DNA polymerase I synthesizes the second-strand fragment. The ends of the double stranded cDNA are repaired, linkers carrying specific restriction enzyme site (for example, Eco RI) are ligated to the ends using *E. coli* DNA ligase. The pool

of the cDNA insert is then ligated into one of a variety of bacteriophage vectors that are commercially available like Lambda-Zap (Stratagene). The procedures are summarized in more detail as follows:

a. *First strand cDNA Synthesis*

Dissolve 10 mg poly(A) + RNA at a concentration of 1 mg/ml in sterile water. Heat the RNA for two to five minutes at 65-70°C then quench immediately on ice. In a separate tube add in the following order (180 ml total) : 20 ml of 5 mM dNTPs (500 μM final each), 40 ml 5× RT buffer (1×final), 10 ml 200 mM DTT (10 mM final), 20 ml 0.5 mg/ml oligo (dT)12-18 (50 mg/ml final), 60 ml deionized water, 10 ml (10 units) RNasin (50 units/ml final). Mix by vortexing, briefly microcentrifuge, and add the mixture to the tube containing the RNA. Add 20 ml (200 U) AMV or MMLV reverse transcriptase for a final concentration of 1000 units/ml in 200 ml. Mix by pipetting up and down several times and remove 10 ml to a separate tube containing 1 ml of α^{32}P dCTP. Typically, incubate both tubes at room temperature for five minutes, then place both tubes at 42°C for one and a half hours. This radiolabeled aliquot is removed to determine incorporation and permit an estimation of recovery; this reaction is stopped by adding 1 ml of 0.5 M EDTA (pH 8.0) and stored frozen at -20°C. The radiolabeled reaction will be used later to estimate the yield and average size of the cDNA inserts. The main reaction is stopped by adding 4 ml of 0.5 M EDTA (pH 8.0) and 200 ml buffered phenol. The mixture is vortexed well, microcentrifuged at room temperature for one minute to separate phases, and the upper aqueous layer is transferred to a fresh tube. Back extract the phenol layer with 1×TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) and pool the aqueous layers from the two extractions. This back extraction of the phenol layer improves the yield. The cDNA is ethanol precipitated using 7.5 M ammonium acetate (final concentration 2.0 to 2.5 M) and 95% ethanol. Place in dry ice/ethanol bath fifteen minutes, warm to 4°C, and microcentrifuge at ten minutes at full speed at 4°C to pellet nucleic acids. The small, yellow-white pellet is then washed with ice-cold 70% ethanol, and microcentrifuged for three minutes at full speed, 4°C. Again, the supernatant is removed and the pellet briefly dried.

b. *Second strand synthesis*

Typically, the pellet from the first-strand synthesis is resuspended in 284 ml water and these reagents are added in the following order (400 ml total): Four ml of 5 mM dNTPs (50 μM final each), 80 ml 5× second-strand buffer (1×final), 12 ml 5 mM β-NAD (150 μM final), 2 ml 10 uCi/ml α-^{32}P dCTP (50 μCi/ml final). Mix by vortexing, briefly microcentrifuge, and add: 4 ml (4 units) RNase H (10 units/ml final), 4 ml (20 units) *E. coli* DNA ligase (50 units/ml final), and 10 ml (100 units) *E. coli* DNA polymerase I (250 units/ml final). Mix by pipetting up and down, briefly microcentrifuge, and incubate twelve to sixteen hours at 14°C. After second-strand synthesis, remove 4 ml of the reaction to determine the yield from the incorporation of the radiolabel into acid-insoluble material. Extract the second-strand synthesis reaction with 400 ml buffered phenol and back extract the phenol phase with 200 ml TE buffer (pH 7.5) as described above. The double stranded cDNA is then ethanol precipitated as described above.

To complete the second-strand synthesis the double-stranded cDNA ends are rendered blunt using a mixture of enzymes. Resuspend the pellet in 42 ml water then add these reagents in the following order (80 ml total): 5 ml 5 mM dNTPs (310 μM final each), 16 ml 5×TA buffer (1×final), 1 ml 5 mM β-NAD (62 μM final). Mix by vortexing, microcentrifuge briefly, and add: 4 ml of 2 mg/ml RNase A (100 ng/ml final), 4 ml (4 units) RNase H (50 units/ml final), 4 ml (20 units) *E. coli* DNA ligase (250 units/ml final) and 4 ml (8 units) T4 DNA polymerase (100 units/ml final). Mix as above and incubate forty-five minutes at 37°C. Add 120 ml TE buffer (pH 7.5) and 1 ml of 10 mg/ml tRNA. Extract with 200 ml buffered phenol and back extract the phenol layer with 100 ml TE buffer as described above. Pool the two aqueous layers and ethanol precipitate as described above.

c. *Addition of linkers to double stranded cDNA*

Combine these reagents in a 0.5 ml microcentrifuge test tube, at room temperature, and in the following order: 100 ng double stranded cDNA, 2 ml linkers/adaptors (10 mM), 2 ml 5× DNA Ligation Buffer, 1 ml T4 DNA Ligase (unit/ml) for a 10 ml final volume. Mix the contents thoroughly with gentle pipetting and spin the tube briefly in a microcentrifuge.

Incubate at either: 16°C overnight; or room temperature (19-23°C) for three to four hours. Inactivate the ligase enzyme by heating the mixture at 70°C for five minutes. This cDNA is typically digested by *Eco* RI to prepare the cohesive ends of the cDNA for ligation into the vector and to cleave linker concatemers. Typically this reaction consists of the 10 ml of the cDNA, 2 ml of 10× *Eco* RI buffer (depending on the company of source), 2 ml of *Eco* RI (10 units/ml) and sterile water to a final volume of 20 ml. The mixture is incubated at 37°C for two to four hours.

d. *Size fractionation of cDNA*

Size exclusion columns are typically used to remove linker molecules and short cDNA fragments (350 bp). For example, a 1-ml Sepharose CL-4B column is prepared in a plastic column plugged with a small piece of sterilized glass wool (a 5 ml plastic pipet will work fine). The column is equilibrated with 0.1 M sodium chloride in 1×TE (10mM Tris, 1 mM EDTA, pH 7.5). The cDNA is then loaded onto the column and 200 µl fractions are collected. 2 µl aliquots of each fraction are analyzed by gel electrophoresis and autoradiography to determine the peak of cDNA elution. Typically, fractions containing the first half of the peak are pooled and purified by ethanol precipitation and resuspending in 10 µl distilled water.

e. *Cloning of cDNA into bacteriophage vector*

Bacteriophage vectors designed for the cloning and propagation of cDNA are provided ready-digested with *Eco* RI and with phosphatased ends from commercial sources (*e.g.*, lambda gt10 from Stratagene). The prepared cDNA is ligated into lambda vectors following manufacturer's instructions. Ligated vector/cDNA molecules are packaged into phage particles using packaging extracts available commercially.

Example 8: Screening of Human cDNA Library

a. *Labeling of cDNA fragments (probes) for library screening*

An RNA probe is used that recognizes nucleotide sequences that are specific to *NaN*, such as 1371-1751 of NaN. Other nucleotide sequences can be developed on the basis of the NaN sequence (Figures 2, 7 & 8) such as nucleotides 765-1160 of the human nucleotide sequence. A *Hind* III/*Bam* HI fragment of *NaN* was inserted in pBluescript (SK+) vector (Stratagene). The sequence of the resulting construct was verified by sequencing. The orientation of the insert is such that the 5' and 3' ends of the construct delineated by the *Hind* III and *Bam* HI restriction enzyme sites, respectively, are proximal to T7 and T3 RNA polymerase promoters, respectively. Digoxigenin-labeled Sense (linearized at the *Hind* III site and transcribed by T7 RNA polymerase) and antisense (linearized at the *Bam* HI site and transcribed by T3 RNA polymerase) transcripts were prepared in vitro using MEGAscript transcription kit (Ambion) according to manufacturer specifications. Briefly, 1 μg linearized template was transcribed with the respective RNA polymerase in a 20 μl final volume containing the following reagents: 1× enzyme mixture containing the respective RNA polymerase and RNase inhibitor and reaction buffer (Ambion), 7.5 mM ATP, GTP and CTP nucleotides, 5.625 mM UTP and 1.725 mM Dig-11UTP (Boehringer Mannheim). *In vitro* transcription was carried out at 37°C for three hours in a water bath. DNA template was removed by adding 1 μl of RNase-free DNase I (2 units/μl) to each reaction and incubating further at 37°C for fifteen minutes. The reaction was then stopped by adding 30 μl nuclease-free water) and 25 μl of LiCl precipitation solution (7.5 M Lithium Chloride, 50 mM EDTA).

The mixture was incubated at -20°C for thirty minutes. The RNA transcripts were pelleted in a microfuge at 13000×g, 4°C for fifteen minutes. The supernatant was removed and the pellet washed once with 100 μl of 75% ethanol. The mixture was re-centrifuged at 13000×g, room temperature for five minutes. The pellet was then air-dried in a closed chamber and subsequently dissolved in 100 ml of RNase-free water. The transcript yield and integrity were determined by comparison to a control DIG-labeled RNA on agarose-formaldehyde gel as described in the DIG/Genius kit according to manufacturer

recommendations (Boehringer Mannheim). Alternatively, a skilled artisan can design radioactive probes for autoradiographic analysis.

Other regions of the rat, mouse or human *NaN* sodium channel cDNA, like 3' untranslated sequences, can also be used as probes in a similar fashion for cDNA library screening or Northern blot analysis. Specifically, a probe is made using commercially available kits, such as the Pharmacia oligo labeling kit, or Genius kit (Boehringer Mannheim).

b. *cDNA library screening*

Recombinant plaques containing full length human homologues of the *NaN* sodium channel are detected using moderate stringency hybridization washes (50-60°C, 5×SSC, thirty minutes), using non-radioactive (see above) or radiolabeled DNA or cRNA *NaN*-specific probes derived from the 3' untranslated or other regions as described above. Libraries are screened using standard protocols (Sambrook *et al.*, (1989) Molecular Cloning - A Laboratory Approach, Cold Spring Harbor Press; Ausubel *et al.*, (1995) Current Protocols in Molecular Biology, Greene Publishing) involving the production of nitrocellulose or nylon membrane filters carrying recombinant phages. The recombinant DNA is then hybridized to *NaN*-specific probes (see above). Moderate stringency washes are carried out.

Plaques which are positive on duplicate filters (*i.e.*, not artefacts or background) are selected for further purification. One or more rounds of screening after dilution to separate the phage are typically performed. Resulting plaques are then purified, DNA is extracted and the insert sizes of these clones characterized. The clones are cross-hybridized to each other using standard techniques (Sambrook *et al.*, (1989) Molecular Cloning - A Laboratory Approach, Cold Spring Harbor Press) and distinct positive clones identified.

Typically, overlapping clones that encode the channel are isolated. Standard cloning techniques are then used to produce a full length cDNA construct that contains any 5' untranslated sequence, the start codon ATG, the coding sequence, a stop codon and any 3' untranslated sequence, a poly A consensus sequence and possibly a poly A run. If overlapping clones do not produce sufficient fragments to assemble a full length cDNA clone, alternative

methods like RACE (PCR-based) could be used to generate the missing pieces or a full length clone.

c. *Characterization of a human homologue full-length clone*

A *NaN*-specific cDNA sequence from a full-length clone is used as a probe in Northern blot analysis to determine the messenger RNA size in human tissue for comparison with the rat and mouse messenger RNA size. Confirmation of biological activity of the cloned cDNA is carried out using methods similar to those described for the rat *NaN*.

Example 9: Polymerase chain reaction (PCR) approaches to clone other full length human *NaN* sodium channels using DNA sequences derived from rat, mouse or human amino acid sequences

Total RNA and poly A+ RNA is isolated from human dorsal root ganglia or trigeminal ganglia or other cranial ganglia from post-mortem human material or foetuses or biopsy/surgical tissues as described above. Preparation of cDNA and PCR-based methods are then used as described previously in Example 1.

Using degenerate PCR primers derived from the rat, mouse or human *NaN*-specific coding sequence (see Figures 2 (SEQ ID NO: 3), 7B (SEQ ID NO: 5), 8B (SEQ ID NO: 8) and 11B (SEQ ID NO: 41)), the cDNA is amplified using the polymerase chain reaction (Saiki *et al.*, (1985) Science 230, 1350-1354). A skilled artisan could utilize the many variables which can be manipulated in a PCR reaction to derive the homologous sequences required. These include, but are not limited to, varying cycle and step temperatures, cycle and step times, number of cycles, thermostable polymerase, and Mg^{2+} concentration. A greater specificity can be achieved using nested primers derived from further conserved sequences from the *NaN* sodium channel.

Amplification is typically performed in 60 µl volume using 1 µl of the first strand cDNA, 0.8 mM of each primer and 1.75 units of Expand Long Template DNA polymerase enzyme mixture (Boehringer Mannheim). Compared to conventional and thermostable DNA polymerases, Expand Long Template enzyme mixture increases the yield of the PCR products

without an increase in non-specific amplification (Barnes, (1994) Proc. Natl. Acad. Sci. USA 91, 2216-2220; Cheng *et al.*, (1994) Proc. Natl. Acad. Sci. USA 91, 5695-5699). The PCR reaction buffer consists of 50 mM Tris-HCl (pH 9.2), 16 mM $(NH_4)_2SO_4$, 2.25 mM $MgCl_2$, 2% (v/v) DMSO and 0.1% Tween 20. As described previously (Dib-Hajj *et al.*, (1996) FEBS Lett. 384, 78-82), amplification is carried out in two stages using a programmable thermal cycler (PTC-200, MJ Research). First, a denaturation step at 94°C for four minutes, an annealing step at 60°C for two minutes and an elongation step at 72°C for ninety seconds. Second, a denaturation step at 94°C for one minute, an annealing step at 60°C for one minute and an elongation step at 72°C for ninety seconds. The second stage is repeated 33 times for a total of 35 cycles, with the elongation step in the last cycle extended to 10 minutes. In addition, control reactions are performed alongside the samples. These should be: (1) all components without cDNA, (negative control) and (2) all reaction components with primers for constitutively expressed product, *e.g*, GAPDH.

The products of the PCR reactions are examined on 1-1.6% (w/v) agarose gels. Bands on the gel (visualized by staining with ethidium bromide and viewing under UV light) representing amplification products of the approximate predicted size are then cut from the gel and the DNA purified.

The resulting DNA may be sequenced directly or is ligated into suitable vectors such as, but not limited to, pCR II (Invitrogen) or pGEMT (Promega). Clones are then sequenced to identify those containing sequence with similarity to the rat, mouse or partial human *NaN* sodium channel sequence.

Example 10: Clone analysis

Candidate clones from Example 9 are further characterized by conventional techniques. The biological activity of expression products is also confirmed using conventional techniques.

Example 11: Isolation of full length *NaN* sequences from human fetal tissues

Commercially available human fetal cDNA libraries and/or cDNA pools are screened with *NaN*-specific primers (by PCR) or probes (library screening) using PCR standard PCR protocols and standard library screening procedures as described above.

Example 12: Northern Blot of rat DRG or Trigeminal Neurons with Fragments of Rat *NaN*

10-30 μg total DRG and/or RNA from DRG or trigeminal (for positive tissues) and cerebral hemisphere, cerebellum and liver (for negative tissues) is electrophoresed in denaturing 1% agarose-formaldehyde gel or agarose-glyoxal gel, and then is transferred to a nylon membrane as described in achieved in multiple steps, as detailed in standard molecular biology manuals (Sambrook *et al.*, (1989) Molecular Cloning - A Laboratory Approach, Cold Spring Harbor Press; Ausubel *et al.*, (1995) Current Protocols in Molecular Biology, Greene Publishing). Radiolabeled (specific activity of $>10^8$ dpm/μg) or Digixoginen-labeled RNA probes are typically used for Northern analysis. An antisense RNA probe (see Example 20, which describes *in situ* hybridization with a *NaN*-specific probe) is created by *in vitro* synthesis from a sense DNA fragment. The membrane carrying the immobilized RNA in wetted with 6×SSC, and the membrane is placed RNA-side-up in a hybridization tube. One ml formamide prehybridization/hybridization solution per 10 cm^2 of membrane is added. Prehybridization and hybridization are usually carried out in glass tubes in a commercial hybridization oven. The tubes are place in a hybridization oven and incubated, with rotation, at 60°C for fifteen minutes to one hour. The desired volume of probe is pipeted into the hybridization tube, and the incubation is continued with rotation overnight at 60°C. The probe concentration in the hybridization solution should be 10 ng/ml if the specific activity is 10^8 dpm/μg or 2 ng/ml if the specific activity is 10^9 dpm/μg (use 2-10 ng/ml of Digixogenin labeled probe).

The hybridization solution is poured off and an equal volume of 2×SSC/0.1% SDS is added. Incubation with rotation for 5 minutes at room temperature is carried out. The wash solution is changed, and this step is repeated. To reduce background, it may be beneficial to double the volume of the wash solutions. The wash solution is replaced with an equal volume

of 0.2×SSC/0.1% SDS and the tube is incubated for five minutes with rotation at room temperature. The wash solution is changed and this step is repeated (this is a low-stringency wash). For moderate or high stringency conditions, further washes are done with wash solutions pre-warmed to moderate (42°C) or high (68°C) temperatures. The final wash solution is removed and the membrane rinsed in 2×SSC at room temperature. Autoradiography is then performed for up to one week. Alternatively, signal is detected using chemiluminescence technology (Amersham) if non-radioactive probes are used. The transcript size is calculated from the signal from the gel in comparison with gel molecular weight standard markers.

Example 13: Tissue specific distribution of NaN by RT-PCR

NaN-specific forward (5' CCCTGCTGCGCTCGGTGAAGAA 3') (SEQ ID NO: 39) and reverse primer (5' GACAAAGTAGATCCCAGAGG 3') (SEQ ID NO: 25), were used in RT-PCR assays using cDNA template prepared from multiple rat. These primers amplify *NaN* sequence between nucleotides 765 and 1156 (392 bp) and are *NaN*-specific as judged by lack of similarity to sequences in the database (using programs like BLASTN from the National Center for Biotechnology Information). Amplification was typically performed in a 60 µl volume using 1 µl of the first strand of cDNA, 0.8 µM of each primer and 1.75 units of Expand Long Template DNA polymerase enzyme mixture (Boehringer Mannheim). Compared to conventional and thermostable DNA polymerases, Expand Long Template enzyme mixture increases the yield of the PCR products without an increase in non-specific amplification (Barnes, (1994) Proc. Natl. Acad. Sci. USA 91, 2216-2220; Cheng *et al.*, (1994) Proc. Natl. Acad. Sci. USA 91, 5695-5699). The PCR reaction buffer consisted of 50 mM Tris-HCl (pH 9.2), 16mM $(NH_4)_2SO_4$, 2.25 mM $MgCl_2$, 2% (v/v) DMSO and 0.1% Tween 20. As described previously (Dib-Hajj *et al.*, (1996) FEBS Lett. 384, 78-82), amplification was carried out in two stages using a programmable thermal cycler (PTC-200, MJ Research). First, a denaturation step is performed at 94°C for four minutes, followed by an annealing step at 60°C for two minutes, and then an elongation step at 72°C for ninety seconds. Second, a denaturation step is performed at 94°C for one minute, followed by an annealing step at 60°C

for one minute, and then an elongation step at 72°C for ninety seconds. The second stage was repeated 33 times for a total of 25-35 cycles, with the elongation step in the last cycle extended to ten minutes.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control to ensure that a lack of *NaN* signals in different tissues was not due to degraded templates or presence of PCR inhibitors. Rat GAPDH sequences were co-amplified using primers which amplify a 66 bp product that corresponds to nucleotides 328-994 (accession number: M17701). The amplified product spans multiple exon/intron splice sites, based on the structure of the human gene (Benham *et al.*, (1987) Nature 328, 275-278). DnaseI treatment was routinely performed prior to reverse transcription to prevent amplification of GAPDH sequences from genomic processed pseudogenes that may have contaminated the total RNA preparation (Ercolani *et al.*, (1988) J. Biol. Chem. 263, 15335-15341).

NaN is primarily and preferentially expressed in DRG and trigeminal ganglia neurons. Figure 4 shows the results of screening by RT-PCR for the expression of *NaN* in various neuronal and non-neuronal tissues. Lanes 1, 2, 4, 9 and 16 show a single amplification product co-migrating with the 400 bp marker, consistent with *NaN*-specific product of 392 bp. Lanes 1, 2, 4, 9 and 16 contain products using DRG, cerebral hemisphere, retina, and trigeminal ganglia, respectively. Using this assay, *NaN* was not detected in cerebellum, optic nerve, spinal cord, sciatic nerve, superior cervical ganglia, skeletal muscle, cardiac muscle, adrenal gland, uterus, liver or kidney (lanes 3, 5-8, and 10-15, respectively). The attenuated *NaN* signal in cerebral hemisphere and retina, and the absence of this signal in the remaining tissues is not due to degraded RNA or the presence of PCR inhibitors in the cDNA templates as comparable GAPDH amplification products were obtained in a parallel set of PCR reaction (data not shown).

Example 14: Transformation of a Host Cell with the *NaN* Coding Sequence

Transformed host cells for the measurement of Na^+ current or intracellular Na^+ levels are usually prepared by co-transfecting constructs into cells such as HEK293 cells with a fluorescent reporter plasmid (pGreen Lantern-1, Life Technologies, Inc.) using the

calcium-phosphate precipitation technique (Ukomadu *et al.*, (1992) Neuron 8, 663-676). HEK293 cells are typically grown in high glucose DMEM (Life Technologies) supplemented with 10% fetal calf serum (Life Technologies). After 48 hours, cells with green fluorescence are selected for recording (Dib-Hajj *et al.*, (1997) FEBS Lett. 416, 11-14).

For preparation of cell lines continuously expressing recombinant channels, the *NaN* construct is cloned into other vectors that carry a selectable marker in mammalian cells. Transfections are carried out using the calcium phosphate precipitation technique (Ukomadu *et al.*, (1992) Neuron 8, 663-676). Human embryonic kidney (HEK-293), chinese hamster ovary (CHO) cells, or other suitable cell lines are grown under standard tissue culture conditions in Dulbeccos's modified Eagle's medium supplemented with 10% fetal bovine serum. The calcium phosphate-DNA mixture is added to the cell culture medium and left for fifteen to twenty hours, after which time the cells are washed with fresh medium. After forty-eight hours, antibiotic (G418) is added to select for cells which have acquired neomycin resistance. After two weeks in G418, 10-20 isolated cell colonies are harvested using sterile 10ml pipette tips. Colonies are grown for another four to seven days, split and subsequently tested for channel expression using whole-cell patch-clamp recording techniques and RT-PCR.

Example 15: Production of NaN specific Antibodies

Antibodies specific to the rat, mouse or human NaN are produced with an immunogenic NaN-specific peptide by raising polyclonal antibodies in rabbits. In one example, the peptide **CGPNPASNKDCFEKEKDSED** (rat amino acids 285-304) (SEQ ID NO: 40) was selected because it fits the criteria for immunogenecity and surface accessibility. This peptide sequence does not match any peptide in the public databases. The underlined cysteine (C) residue was changed to Alanine (A) to prevent disulfide bond formation. This amino acid change is not expected to significantly affect the specificity of the antibodies.

Peptide synthesis, rabbit immunization, and affinity purification of the antipeptide antibodies were performed using standard procedures. Purified antibodies were then tested on

DRG neurons in culture. Immunostaining procedures using these antibodies before and after blocking with excess peptide were performed according to standard procedures.

DRG neurons after sixteen to twenty-four hours in culture were processed for immunocytochemical detection of NaN protein as follows. Coverslips were washed with complete saline solution (137 mM NaCl, 5.3 mM KCI, 1 ITIM M902 25 mM sorbitol, 10 mM HEPES, 3 mM $CaCl_2$ (pH 7.2)), fixed with 4% paraformaldehyde in 0. 14 M phosphate buffer for ten minutes at 4°C, washed with three five minutes with phosphate-buffered saline (PBS), and blocked with PBS containing 20% normal goat serum, 1% bovine serum albumin and 0. 1 % Triton X- 100 for fifteen minutes. The coverslips were incubated in anti-NaN antibody (1:100 dilution) at 4°C overnight. Following overnight incubation, coverslips were washed extensively in PBS and then incubated with goat anti-rabbit IgG-conjugated to Cy3 (1:3000; Amersham) for two hours at room temperature. The coverslips were rinsed with PBS and mounted onto glass slides with Aqua-poly-mount. The neurons were examined with a Leitz Aristoplan light microscope equipped with epifluorescence and images were captured with a Dage DC330T color camera and Scion CG-7 color PCI frame grabber (see Figure 7).

Example 16: NaN expression is altered in a neuropathic pain model

The CCI model of neuropathic pain was used to study the plasticity of sodium channel expression in DRG neurons. Twenty two adult, femal Sprague-Dawley rats, weighing 240-260 g were anesthetized with pentobarbital sodium (50 mg/kg ip) and the right sciatic nerve exposed at the mid-thigh. Four chromic gut (4-0) ligatures were tied loosely around the nerve as described by Bennett & Xie, (1988) Pain 33, 87-107. The incision site was closed in layers and a bacteriostatic agent administered intramuscularly.

Previous studies have shown that transection of the sciatic nerve induces dramatic changes in sodium currents of axotomized DRG neurons, which is paralleled by significant changes to transcripts of various sodium channels expressed in these neurons. Sodium currents that are TTX-R and the transcripts of two TTX-R sodium channels (SNS/PN3 and NaN) are significantly attenuated while a rapidly repriming silent TTX-S current emerges and the transcript of α-III sodium channel, which produces a TTX-S current, is up-regulated. We

have discovered that CCI-induced changes in DRG neurons, fourteen days post-surgery, mirror those of axotomy. Transcripts of NaN and SNS, the two sensory neuron-specific TTX-R channels, are significantly down-regulated as is the TTX-R sodium current, while transcripts of the TTX-S α-III sodium channel are up-regulated, in small diameter DRG neurons. These changes may be partly responsible for making DRG neurons hyperexcitable, that contributes to the hyperalgesia that results from this injury.

Example 17: Assays for agents which modulate the activity of the NaN channel using patch clamp methods

Cells lines expressing the cloned Na^+ channel are used to assay for agents which modulate the activity of the NaN channel, *e.g.*, agents which block or inhibit the channel or enhance channel opening. Since the channel activation is voltage dependent, depolarizing conditions may be used for observation of baseline activity that is modified by the agent to be tested. Depolarization may be achieved by any means available, for example, by raising the extracellular potassium ion concentration to about 20 to 40 nM, or by repeated electrical pulses.

The agent to be tested is incubated with HEK 293 or other transformed cells that express the Na^+ channel (Dib-Hajj *et al.*, (1997) FEBS Lett. 416, 11-14). After incubation for a sufficient period of time, the agent induced changes in Na^+ channel activity can be measured by patch-clamp methods (Hamill *et al.*, (1981) Pflügers Arch. 391, 85-100). Data for these measurements are acquired on a MacIntosh Quadra 950, or similar computer, using a program such as Pulse (v 7.52, HEKA, German). Fire-polished electrodes (0.8-1.5 MW) are fabricated from capillary glass using a Sutter P-87 puller or a similar instrument. Cells are usually only considered for analysis if initial seal resistance is <5 Gohm, they have high leakage currents (holding current <0.1 nA at -80 mV), membrane blebs, and an access resistance <5 Mohm. Access resistance is monitored and data is not used if resistance changes occur. Voltage errors are minimized using series resistance compensation and the capacitance artifact will be canceled as necessary using computer-controlled amplifier circuitry or other similar methods.

For comparisons of the voltage dependence of activation and inactivation, cells with a maximum voltage error of <10 mV after compensation are usually used. Linear leak subtraction is used for voltage clamp recordings. Membrane currents are typically filtered at 5 KHz and sampled at 20 KHz. The pipette solution contains a standard solution such as: 140 mM CsF, 2 mM $MgCl_2$, 1 mM EGTA, and 10 mM Na-HEPES (pH 7.3). The standard bathing solution is a standard solution such as 140 mM NaCl, 3 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES, and 10 mM glucose (pH 7.3).

Tetrodotoxin (TTX)-resistant and TTX-sensitive Na^+ currents are measured by exposure to appropriate concentrations of TTX and/or by pre-pulse protocols which distinguish between TTX-sensitive and TTX-resistant currents on the basis of their distinct steady-state inactivation properties (Cummins & Waxman (1997) J. Neurophysiol. 17, 3503-3514; Sontheimer & Waxman, (1992) J. Neurophysiol. 68, 1001-1011).

Data are collected using standard pulse protocols and are analyzed to measure sodium current properties that include voltage-dependence, steady-state characteristics, kinetics, and re-priming. Measurements of current amplitude and cell capacitance provides an estimate of Na^+ current density, thereby permitting comparisons of channel density under different conditions (Cummins & Waxman (1997) J. Neurophysiol. 17, 3503-3514,30). Cells are studied in the current clamp mode to study patterns of spontaneous and evoked action potential generation, threshold for firing, frequency response characteristics, and response to de- and hyperpolarization, and other aspects of electrogenesis (Sontheimer & Waxman, (1992) J. Neurophysiol. 68, 1001-1011). These measurements are carried out both in control cells expressing *NaN* and in cells expressing *NaN* that also have been exposed to the agent to be tested.

Example 18: Assays for agents which modulate the activity of the NaN channel by the measurement of Intracellular Sodium [Na^+]

The agent to be tested is incubated with cells exhibiting NaN channel activity. After incubation for a sufficient period of time, the agent induced changes in Na^+ channel are measured by ratiometric imaging of $[Na^+]_i$ using SBFI. In this method, cytosolic-free Na^+ is

measured using an indicator for Na^+, such as SBFI (sodium-binding benzofuran isophthalate (Harootunian *et al.*, (1989) J. Biol. Chem. 264, 19458-19467)); or a similar dye. Cells are first loaded with the membrane-permeable acetoxymethyl ester form of SBFI (SBFI/AM) or a similar dye (usually dissolved in dimethyl sulfoxide (DMSO) at a stock concentration of 10 mM). Recordings are obtained on the stage of a microscope using a commercially available ratiometric imaging setup (*e.g.*, from Georgia Instruments). Excitation light is provided at appropriate wavelengths (*e.g.*, 340:385 nm). Excitation light is passed to the cells through a dichroic reflector (400 nm) and emitted light above 450 nm was collected. Fluorescence signals are amplified, *e.g.*, by an image intensifier (GenIISyS) and collected with a CCD camera, or similar device, interfaced to a frame grabber. To account for fluorescence rundown, the fluorescence ratio 340:385 is used to assay cytosolic-free Na^+.

For calibration of SBFI's fluorescence, cells are perfused with calibration solutions containing known Na^+ concentrations (typically 0 and 30 mM, or 0, 30, and 50 mM $[Na^+]$, and gramicidin and monensin. As reported by Rose and Ransom (Rose & Ransom, (1996) J. Physiol. (Lond) 491, 291-305), the 345/390 nm fluorescence ratio of intracellular SBFI changes monotonically with changes in $[Na^+]_i$. Experiments are repeated on multiple (typically at least four) different coverslips, providing statistically significant measurements of intracellular sodium in control cells, and in cells exposed to various concentrations of agents that may block, inhibit or enhance the activity of the channel. Use of this method is illustrated in Sontheimer *et al.*, (1994) J. Neurosci. 14, 2464-2475.

Example 19: Assays for agents which modulate the activity of the NaN channel by scintigraphic imaging

Cells lines expressing the cloned Na^+ channel are used to assay for agents which modulate the activity of the NaN channel, *e.g.*, agents which block the channel or enhance channel opening. For example, the agent to be tested is incubated with HEK 293 or other transformed cells that express the Na^+ channel (Dib-Hajj *et al.*, (1997) FEBS Lett. 416, 11-14). After incubation for a sufficient period of time, the agent induced changes in Na^{++} channel activity are detected by the measurement of Na^+ influx by isotopic methods. ^{22}Na is a

gamma emitter and can be used to measure Na^+ flux (Kimelberg & Waltz, (1988) The Neuronal Microenvironment (Boulton *et al.*, editors) Humana Press) and $^{86}Rb^+$ can be used to measure Na^+/K^+ATPase activity which provides a measure of Na channel activity (Sontheimer *et al.*, (1994) J. Neurosci. 14, 2464-2475) $^{86}Rb^+$ ions are taken up by the Na^+/K+ATPase like K+ ions, but have the advantage of a much longer half-life than $^{42}K^+$ (Kimelberg & Mayhew (1975) J. Biol. Chem. 250, 100-104). Thus, measurement of the unidirectional ouabain-sensitive $^{86}Rb^+$ uptake provides a quantitative method for assaying Na^+/K^+-ATPase activity which follows action potentials.

Following incubation of cell expressing *NaN* to the isotope, the cellular content of the isotope is measured by liquid scintillation counting or a similar method, and cell protein is determined using a method such as the bicinchoninic acid protein assay (Smith *et al.*, (1985) Anal. Biochem. 150, 76-85) following the modifications (Goldschmidt & Kimelberg (1989) Anal. Biochem. 177, 41-45) for cultured cells. ^{22}Na and $^{86}Rb^+$ fluxes are determined in the presence and absence of agents that may block, inhibit, or enhance Na^+. This permits determination of the actions of these agents on *NaN*.

Example 20: *In situ* hybridization

a. *Probes*

Probes are prepared as described above in Example 5.

b. *DRG Neuron Culture*

Cultures of DRG neurons from adult rats were established as described previously (Rizzo *et al.*, (1994) J. Neurophysiol. 72, 2796-2815). Briefly, lumbar ganglia (L4, L5) from adult Sprague Dawley female rats were freed from their connective sheaths and incubated sequentially in enzyme solutions containing collagenase and then papain. The tissue was triturated in culture medium containing 1:1 Dulbecco's modified Eagle's medium (DMEM) and Hank's F12 medium and 10% fetal calf serum, 1.5 mg/ml trypsin inhibitor, 1.5 mg/ml bovine serum albumin, 100 units/ml penicillin and 0.1 mg/ml streptomycin and plated at a density of 500-1000 cells/mm^2 on polyornithine/laminin coated coverslips. The cells were

maintained at 37°C in a humidified 95% air/5% CO_2 incubator overnight and then processed for *in situ* hybridization cytochemistry as described previously (Black *et al.*, (1994) Brain Res. Mol. Brain Res. 23, 235-245; Zur *et al.*, (1995)Brain Res. Mol. Brain Res. 30, 97-105). Trigeminal ganglia can be cultured by a skilled artisan using similar methods.

c. *Tissue Preparation*

Adult female Sprague Dawley rats were deeply anesthetized, *e.g.*, with chloral hydrate and perfused through the heart, first with a phosphate-buffered saline (PBS) solution and then with a 4% paraformaldehyde in 0.14 M Sorensen's phosphate buffer (pH 7.4) at 4°C. Following perfusion fixation, dorsal root ganglia at levels L4 and L5 and trigeminal ganglia were collected and placed in fresh fixative at 4°C. After two to four hours, the tissue was transferred to a solution containing 4% paraformaldehyde and 30% sucrose in 0.14 M phosphate buffer and stored overnight at 4°C. Fifteen μm sections were cut and placed on poly-L-lysine-coated slides. The slides were processed for *in situ* hybridization cytochemistry as previously described (Waxman *et al.*, (1994) J. Neurophysiol. 72, 466-470; Black *et al.*, (1994) Brain Res. Mol. Brain Res. 23, 235-245). Following *in situ* hybridization cytochemistry, the slides were dehydrated, cleared and mounted with Permount. The results are shown in Figure 5.

Sections of DRG hybridized with *NaN* sense riboprobe showed no specific labeling (panel C, Figure 5). In DRG (panel A, Figure 5) and trigeminal (panel B) sections hybridized with a *NaN* antisense riboprobe, with the *NaN* signal present in most small (<30 mm diam.) neurons; in contrast, most large (>30 mm diam.) neurons did not exhibit *NaN* hybridization signal. Sections of spinal cord, cerebellum and liver hybridized with an antisense *NaN* riboprobe showed no specific signal (panels D, E and F respectively).

Example 21: Microsatellite Sequences

The following are the murine intronic microsatellite sequences. These microsatellites may be polymorphic in the human SCN11a gene and could be used as markers to screen for

mutant alleles that are associated with a disease. Such screening methods, for instance, hybridization or amplification assays, are readily available.

Intron 4; microsatellite is dTdG (SEQ ID NO: 29)

AGTTTAATGTTGAGTGAATTGTGGTGGTGATTTCCCACTTGAGGCCTTTGTGTTAA
AGCCCAATGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT
GTGTGGTTGGGGGGTGGTGGCAGAGTCTGGTATTGGTAAGGTGAGAGCAATCCCA
GAACGTCCACCTGCTCTTCCATTTTATTAATCAGGCAGGCCTCT

Intron 5; microsatellite is dCdTdG (dNdG2)$_x$ (X5-30) (SEQ ID NO: 30)

GTAAGCCACTGGCTCTTAACTAAAATGCTCGTTGGCATTAGAACATTTCTGAGCTG
GGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGT
GATGGTGGTGGTGGAGGTGGNGGTGGAGGTGGTGGCTGTGGTGGTGGNGGTGGT
GGTGGTGGTGGANGTGGANGTGGTGGCGTGGTGGTGGNGGTGGTGGTGGAGGTG
GTGGCTGTGGTGGTNGTGGTGGC

Intron 6; microsatellite is dCdA (SEQ ID NO: 31)

TGTGCATGCTTGATTCCCAGCTCCTATGGTCTGATTACTCGGTCCTTAGGAGCAAG
GCCAGACTGTCCACCCTGACACACACACACACACACACACACACACACACACAC
ACACACACACACAGTGTAGAGAATTACCTCATTCTTGGAGTTTCTCTGGAAAAGG
AATGTCTCAAAGCCAAGTTCACAGAGCAACAGCTG

Intron 8; 5' microsatellite is dTdC followed by a stretch of dT (SEQ ID NO: 32)

TGTTAGAAACTCTAAGACAATGAAGCACCATGCTGGAAATAAGAGCACAAACTCT
TTCTTCATGCATTACCCACTGCTTGTGCTTTCACCTTAGTGCTCGTGCTCTCTCTTT
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTTTT
TTTTTTTTTT

Intron 8; 3' microsatellite is dCdA (SEQ ID NO: 33)

CACACACACACACACACACACACACACACACACACACACAGAGAAACACTGTCG
CAGTCATACATATAAAGATAAATACATCTTAAAAAAAGAACCATGTGATTG
AGTTATAAAATATTCCAACTTAT

Intron 10B; microsatellite is dCdA followed, three nucleotides downstream by $dCdA_3$ (SEQ ID NO: 34)

AGGTCATTTCCTCTGCAGTGTGCTTGGCAGGAAAAACTTCCTGGCTATTCAAGTCA
GTGCCCTGCTTGATCATCCATGTATCACACACACACAAAACAAACAAACAAACAA
ACAAAACCCTGGGGAAGAAGGAAGAGGTTAAGCACATAGGCAGAGAGCAGCCA
GGCTGACTCAGAGCAAACACCTGATCATTCTTCCAT

Intron 12; microsatellite is dPydG (dT/dCdG) (SEQ ID NO: 35)

GTGCTGGGATCAAAGGCGTGCGCCGCCACCACGCCCGGCCCCTTTTTATGTTTCA
AATTTACTTTTATCATGTGCACGTGTGTGGGTGCGTGCATGTGTGTGCGTGCGTGT
GCGTGTGNGTGTGNGTGTGTGTGTGTGTGTGTGTGTGTGTGTG

Intron 14; microsatellite is dCdA (SEQ ID NO: 36)

CACACACACACACACACACACACACACACACACACACACACACACACACACACA
CACTTGCATCTTTGAGTTAATTGGATAGGCTGAGTCTTACACCGGAATCATACTGT
TGC

Intron 15A; microsatellite is dCdA (SEQ ID NO: 37)

CCAATGAGAGACTCTTGTCTCAAAAAAGCCATGGTGTCCAGATCCTGAGGAATAA
CACCTAAGAATGTGCTCTGGCCTGAAAACACACACACACACACACACACACACAC
ACACACACACAGTTTTATTTATTTATTTAAAAAAATATGTCTCTAGGCATTGCTGA
AATGTCTCCTACAGGATTAAGTCAACCAGAGCCA

It should be understood that the foregoing discussion and examples merely present a detailed description of certain preferred embodiments. It will be apparent to those of ordinary skill in the art that various modification and equivalents can be made without departing from the spirit and scope of the invention. The documents cited and referred to in this patent specification are hereby incorporated by reference in their entirety.

CLAIMS

1. An isolated nucleic acid molecule selected from the group consisting of a nucleic acid molecule comprising SEQ ID NO: 41, a nucleic acid molecule which encodes the amino acid sequence of SEQ ID NO: 42, a nucleic acid molecule which encodes an allelic variant of SEQ ID NO:42, a nucleic acid molecule which encodes a human protein exhibiting at least about 76% amino acid sequence identity to SEQ ID NO:42 and a nucleic acid molecule that hybridizes to one of the foregoing sequences under stringent conditions.

2. The isolated nucleic acid of claim 1, wherein the nucleic acid encodes a voltage gated Na^+ channel that is preferentially expressed in dorsal root ganglia or trigeminal ganglia.

3. The isolated nucleic acid of claim 2, wherein the nucleic acid encodes the human NaN sodium channel.

4. An expression vector comprising the isolated nucleic acid of any one of claims 1 to 3, alone or together with appropriate regulatory and expression control elements.

5. A host cell transformed with the expression vector of claim 4.

6. A Na^+ channel encoded by an isolated nucleic acid molecule of any one of claims 1 to 3.

7. The Na^+ channel of claim 6, comprising the amino acid sequence of SEQ ID NO:42.

8. An isolated protein consisting of the amino acid sequence of SEQ ID NO: 42 or a peptide fragment thereof.

9. A protein comprised within a membrane fragment isolated from the host cell of claim 5.

10. A method to identify an agent that modulates the activity of the Na^+ channel of claim 6, comprising the steps of bringing the agent into contact with a cell that expresses the Na^+ channel on its surface and measuring any resultant changes in the sodium current, resultant change in membrane potential or change in intracellular Na^+.

11. The method of claim 10, wherein the measuring step is accomplished by voltage clamp measurements or measurement of membrane potential.

12. The method of claim 10, wherein the measuring step is accomplished by measuring the level of intracellular sodium.

13. The method of claim 10, wherein the measuring step is accomplished by measuring sodium influx.

14. The method of claim 13, wherein the sodium influx is measured using ^{22}Na or ^{86}Rb.

15. A method to identify an agent that modulates the transcription or translation of mRNA encoding the Na^+ channel of claim 6, comprising the steps of bringing the agent into contact with a cell that expresses the Na^+ channel and measuring the resultant level of expression of the Na^+ channel.

16. A method to treat pain, paraesthesia and/or hyperexcitability phenomena in an animal or human subject by administering an effective amount of an agent that alters Na^+ current flow through NaN channels in DRG or trigeminal neurons.

17. A method to treat pain, paraesthesia and/or hyperexcitability phenomena in an animal or human subject by administering an effective amount of an agent that modulates the transcription or translation of mRNA encoding the Na^+ channel of claim 6.

18. An isolated nucleic acid that is antisense to the nucleic acid of claim 1 and of sufficient length to modulate the expression of NaN channel in a cell containing the mRNA.

19. A scintigraphic method to image the loci of pain generation or provide a measure of the level of pain associated with DRG or trigeminal neuron mediated hyperexcitability in an animal or human subject by administering labeled monoclonal antibodies or other labeled ligands specific for the human NaN Na^+ channel.

20. A method to identify tissues, cells or cell types that express the human NaN sodium channel, comprising the step of detecting human NaN on the cell surface or intracellularly.

21. A method to identify tissues, cells or cell types that express human NaN comprising the step of detecting the presence therein of human NaN encoding mRNA.

22. A method of producing a transformed cell that expresses an exogenous NaN encoding nucleic acid, comprising the step of transforming the cell with an expression vector comprising the isolated nucleic acid of any of claims 1 to 3, together with appropriate regulatory or expression control elements.

23. An isolated antibody specific for the human NaN channel or polypeptide fragment thereof.

24. The isolated antibody of claim 23, wherein the antibody is labeled.

25. A method of producing recombinant NaN protein, comprising the step of culturing the transformed host of claim 5 under conditions in which the NaN sodium channel or protein is expressed.

26. A therapeutic composition comprising an effective amount of an agent capable of altering, such as by increasing or decreasing, the rapidly repriming current flow in axotomized, inflamed or otherwise injured DRG neurons.

27. A method to treat acute pain or acute or chronic neuropathic or inflammatory pain and hyperexcitability phenomena in an animal or a human patient by administering the therapeutic composition of claim 26.

28. A method to screen candidate compounds for use in treating pain and hyperexcitability phenomena by testing their ability to upregulate or downregulate the NaN channel mRNA in axotomized, inflamed or otherwise injured DRG neurons.

29. A chimeric NaN channel.

30. A chimeric channel of claim 29, wherein at least one human domain has been substituted with the corresponding domain from the NaN channel of another species.

31. A chimeric channel of claim 30, wherein the species is rat or mouse.

32. A nucleic acid molecule encoding a chimeric NaN channel of any one of claims 29 through 31.

33. An NaN channel protein comprising a positively charged amino acid at a position corresponding to residue 670 of SEQ ID NO:42.

34. An NaN channel protein of claim 33, wherein the positively charged amino acid is arginine.

35. An isolated nucleic acid molecule encoding a channel protein of either of claims 33 or 34.

36. A therapeutic composition of claim 26, further comprising at least one second agent that modulates a channel in primary sensory neurons.

37. A therapeutic composition of claim 36, wherein the composition comprises agents which modulate NaN and at least one channel selected from the group consisting of PN1/hNE and SNS/PN3.

SEQUENCE LISTING

<110> Yale University
Dib-Hajj, Sulayman
Waxman, Stephen G.

<120> Modulation of Sodium Channels in Dorsal Root Ganglia

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10 15 20

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    135                 140                 145

atg gag aga agt ttc gac aac gac att ccc gaa tac gtc ttc att ggg   535
Met Glu Arg Ser Phe Asp Asn Asp Ile Pro Glu Tyr Val Phe Ile Gly
150                 155                 160                 165

att tat att tta gaa gct gtg att aaa ata ttg gca aga ggc ttc att   583
Ile Tyr Ile Leu Glu Ala Val Ile Lys Ile Leu Ala Arg Gly Phe Ile
                170                 175                 180

gtg gat gag ttt tcc ttc ctc cga gat ccg tgg aac tgg ctg gac ttc   631
Val Asp Glu Phe Ser Phe Leu Arg Asp Pro Trp Asn Trp Leu Asp Phe
            185                 190                 195

att gtc att gga aca gcg atc gca act tgt ttt ccg ggc agc caa gtc   679
Ile Val Ile Gly Thr Ala Ile Ala Thr Cys Phe Pro Gly Ser Gln Val
        200                 205                 210

aat ctt tca gct ctt cgt acc ttc cga gtg ttc aga gct ctg aag gcg   727
Asn Leu Ser Ala Leu Arg Thr Phe Arg Val Phe Arg Ala Leu Lys Ala
    215                 220                 225

att tca gtt atc tca ggt ctg aag gtc atc gta ggt gcc ctg ctg cgc   775
Ile Ser Val Ile Ser Gly Leu Lys Val Ile Val Gly Ala Leu Leu Arg
230                 235                 240                 245

tcg gtg aag aag ctg gta gac gtg atg gtc ctc act ctc ttc tgc ctc   823
Ser Val Lys Lys Leu Val Asp Val Met Val Leu Thr Leu Phe Cys Leu
                250                 255                 260

agc atc ttt gcc ctg gtc ggt cag cag ctg ttc atg gga att ctg aac   871
Ser Ile Phe Ala Leu Val Gly Gln Gln Leu Phe Met Gly Ile Leu Asn
            265                 270                 275

cag aag tgt att aag cac aac tgt ggc ccc aac cct gca tcc aac aag   919
Gln Lys Cys Ile Lys His Asn Cys Gly Pro Asn Pro Ala Ser Asn Lys
        280                 285                 290
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gat tgt ttt gaa aag gaa aaa gat agc gaa gac ttc ata atg tgt ggt   967
Asp Cys Phe Glu Lys Glu Lys Asp Ser Glu Asp Phe Ile Met Cys Gly
    295                 300                 305

acc tgg ctc ggc agc aga ccc tgt ccc aat ggt tct acg tgc gat aaa  1015
Thr Trp Leu Gly Ser Arg Pro Cys Pro Asn Gly Ser Thr Cys Asp Lys
310                 315                 320                 325

acc aca ttg aac cca gac aat aat tat aca aag ttt gac aac ttt ggc  1063
Thr Thr Leu Asn Pro Asp Asn Asn Tyr Thr Lys Phe Asp Asn Phe Gly
                330                 335                 340

tgg tcc ttt ctc gcc atg ttc cgg gtt atg act caa gac tcc tgg gag  1111
Trp Ser Phe Leu Ala Met Phe Arg Val Met Thr Gln Asp Ser Trp Glu
            345                 350                 355

agg ctt tac cga cag atc ctg cgg acc tct ggg atc tac ttt gtc ttc  1159
Arg Leu Tyr Arg Gln Ile Leu Arg Thr Ser Gly Ile Tyr Phe Val Phe
        360                 365                 370

ttc ttc gtg gtg gtc atc ttc ctg ggc tcc ttc tac ctg ctt aac cta  1207
Phe Phe Val Val Val Ile Phe Leu Gly Ser Phe Tyr Leu Leu Asn Leu
    375                 380                 385

acc ctg gct gtt gtc acc atg gct tat gaa gaa cag aac aga aat gta  1255
Thr Leu Ala Val Val Thr Met Ala Tyr Glu Glu Gln Asn Arg Asn Val
390                 395                 400                 405

gct gct gag aca gag gcc aag gag aaa atg ttt cag gaa gcc cag cag  1303
Ala Ala Glu Thr Glu Ala Lys Glu Lys Met Phe Gln Glu Ala Gln Gln
                410                 415                 420

ctg tta agg gag gag aag gag gct ctg gtt gcc atg gga att gac aga  1351
Leu Leu Arg Glu Glu Lys Glu Ala Leu Val Ala Met Gly Ile Asp Arg
            425                 430                 435

agt tcc ctt aat tcc ctt caa gct tca tcc ttt tcc ccg aag aag agg  1399
Ser Ser Leu Asn Ser Leu Gln Ala Ser Ser Phe Ser Pro Lys Lys Arg
        440                 445                 450

aag ttt ttc ggt agt aag aca aga aag tcc ttc ttt atg aga ggg tcc  1447
Lys Phe Phe Gly Ser Lys Thr Arg Lys Ser Phe Phe Met Arg Gly Ser
    455                 460                 465

aag acg gcc caa gcc tca gcg tct gat tca gag gac gat gcc tct aaa  1495
Lys Thr Ala Gln Ala Ser Ala Ser Asp Ser Glu Asp Asp Ala Ser Lys
470                 475                 480                 485

aat cca cag ctc ctt gag cag acc aaa cga ctg tcc cag aac ttg cca  1543
Asn Pro Gln Leu Leu Glu Gln Thr Lys Arg Leu Ser Gln Asn Leu Pro
                490                 495                 500

gtg gat ctc ttt gat gag cac gtg gac ccc ctc cac agg cag aga gcg  1591
Val Asp Leu Phe Asp Glu His Val Asp Pro Leu His Arg Gln Arg Ala
            505                 510                 515
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ctg agc gct gtc agt atc tta acc atc acc atg cag gaa caa gaa aaa   1639
Leu Ser Ala Val Ser Ile Leu Thr Ile Thr Met Gln Glu Gln Glu Lys
        520                 525                 530

ttc cag gag cct tgt ttc cca tgt ggg aaa aat ttg gcc tct aag tac   1687
Phe Gln Glu Pro Cys Phe Pro Cys Gly Lys Asn Leu Ala Ser Lys Tyr
    535                 540                 545

ctg gtg tgg gac tgt agc cct caa tgg ctg tgc ata aag aag gtc ctg   1735
Leu Val Trp Asp Cys Ser Pro Gln Trp Leu Cys Ile Lys Lys Val Leu
550                 555                 560                 565

cgg acc atc atg acg gat ccc ttt act gag ctg gcc atc acc atc tgc   1783
Arg Thr Ile Met Thr Asp Pro Phe Thr Glu Leu Ala Ile Thr Ile Cys
                570                 575                 580

atc atc atc aat acc gtt ttc tta gcc gtg gag cac cac aac atg gat   1831
Ile Ile Ile Asn Thr Val Phe Leu Ala Val Glu His His Asn Met Asp
            585                 590                 595

gac aac tta aag acc ata ctg aaa ata gga aac tgg gtt ttc acg gga   1879
Asp Asn Leu Lys Thr Ile Leu Lys Ile Gly Asn Trp Val Phe Thr Gly
        600                 605                 610

att ttc ata gcg gaa atg tgt ctc aag atc atc gcg ctc gac cct tac   1927
Ile Phe Ile Ala Glu Met Cys Leu Lys Ile Ile Ala Leu Asp Pro Tyr
    615                 620                 625

cac tac ttc cgg cac ggc tgg aat gtt ttt gac agc atc gtg gcc ctc   1975
His Tyr Phe Arg His Gly Trp Asn Val Phe Asp Ser Ile Val Ala Leu
630                 635                 640                 645

ctg agt ctc gct gat gtg ctn tac aac aca ctg tct gat aac aat agg   2023
Leu Ser Leu Ala Asp Val Xaa Tyr Asn Thr Leu Ser Asp Asn Asn Arg
                650                 655                 660

tct ttc ttg gct tcc ctc aga gtg ctg agg gtc ttc aag tta gcc aaa   2071
Ser Phe Leu Ala Ser Leu Arg Val Leu Arg Val Phe Lys Leu Ala Lys
            665                 670                 675

tcc tgg ccc acg tta aac act ctc att aag atc atc ggc cac tcc gtg   2119
Ser Trp Pro Thr Leu Asn Thr Leu Ile Lys Ile Ile Gly His Ser Val
        680                 685                 690

ggc gcg ctt gga aac ctg act gtg gtc ctg act atc gtg gtc ttc atc   2167
Gly Ala Leu Gly Asn Leu Thr Val Val Leu Thr Ile Val Val Phe Ile
    695                 700                 705

ttt tct gtg gtg ggc atg cgg ctc ttc ggc acc aag ttt aac aag acc   2215
Phe Ser Val Val Gly Met Arg Leu Phe Gly Thr Lys Phe Asn Lys Thr
710                 715                 720                 725

gcc tac gcc acc cag gag cgg ccc agg cgg cgc tgg cac atg gat aat   2263
Ala Tyr Ala Thr Gln Glu Arg Pro Arg Arg Arg Trp His Met Asp Asn
                730                 735                 740
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ttc tac cac tcc ttc ctg gtg gtg ttc cgc atc ctc tgt ggg gaa tgg   2311
Phe Tyr His Ser Phe Leu Val Val Phe Arg Ile Leu Cys Gly Glu Trp
            745                 750                 755

atc gag aac atg tgg ggc tgc atg cag gat atg gac ggc tcc ccg ttg   2359
Ile Glu Asn Met Trp Gly Cys Met Gln Asp Met Asp Gly Ser Pro Leu
        760                 765                 770

tgc atc att gtc ttt gtc ctg ata atg gtg atc ggg aag ctt gtg gtg   2407
Cys Ile Ile Val Phe Val Leu Ile Met Val Ile Gly Lys Leu Val Val
    775                 780                 785

ctt aac ctc ttc att gcc ttg ctg ctc aat tcc ttc agc aat gag gag   2455
Leu Asn Leu Phe Ile Ala Leu Leu Leu Asn Ser Phe Ser Asn Glu Glu
790                 795                 800                 805

aag gat ggg agc ctg gaa gga gag acc agg aaa acc aaa gtg cag cta   2503
Lys Asp Gly Ser Leu Glu Gly Glu Thr Arg Lys Thr Lys Val Gln Leu
                810                 815                 820

gcc ctg gat cgg ttc cgc cgg gcc ttc tcc ttc atg ctg cac gct ctt   2551
Ala Leu Asp Arg Phe Arg Arg Ala Phe Ser Phe Met Leu His Ala Leu
            825                 830                 835

cag agt ttt tgt tgc aag aaa tgc agg agg aaa aac tcg cca aag cca   2599
Gln Ser Phe Cys Cys Lys Lys Cys Arg Arg Lys Asn Ser Pro Lys Pro
        840                 845                 850

aaa gag aca aca gaa agc ttt gct ggt gag aat aaa gac tca atc ctc   2647
Lys Glu Thr Thr Glu Ser Phe Ala Gly Glu Asn Lys Asp Ser Ile Leu
    855                 860                 865

ccg gat gcg agg ccc tgg aag gag tat gat aca gac atg gct ttg tac   2695
Pro Asp Ala Arg Pro Trp Lys Glu Tyr Asp Thr Asp Met Ala Leu Tyr
870                 875                 880                 885

act gga cag gcc ggg gct ccg ctg gcc cca ctc gca gag gta gag gac   2743
Thr Gly Gln Ala Gly Ala Pro Leu Ala Pro Leu Ala Glu Val Glu Asp
                890                 895                 900

gat gtg gaa tat tgt ggt gaa ggc ggt gcc cta ccc acc tca caa cat   2791
Asp Val Glu Tyr Cys Gly Glu Gly Gly Ala Leu Pro Thr Ser Gln His
            905                 910                 915

agt gct gga gtt cag gcc ggt gac ctc cct cca gag acc aag cag ctc   2839
Ser Ala Gly Val Gln Ala Gly Asp Leu Pro Pro Glu Thr Lys Gln Leu
        920                 925                 930

act agc ccg gat gac caa ggg gtt gaa atg gaa gta ttt tct gaa gaa   2887
Thr Ser Pro Asp Asp Gln Gly Val Glu Met Glu Val Phe Ser Glu Glu
    935                 940                 945

gat ctg cat tta agc ata cag agt cct cga aag aag tct gac gca gtg   2935
Asp Leu His Leu Ser Ile Gln Ser Pro Arg Lys Lys Ser Asp Ala Val
950                 955                 960                 965
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agc atg ctc tcg gaa tgc agc aca att gac ctg aat gat atc ttt aga   2983
Ser Met Leu Ser Glu Cys Ser Thr Ile Asp Leu Asn Asp Ile Phe Arg
                970                 975                 980

aat tta cag aaa aca gtt tcc ccc aaa aag cag cca gat aga tgc ttt   3031
Asn Leu Gln Lys Thr Val Ser Pro Lys Lys Gln Pro Asp Arg Cys Phe
            985                 990                 995

ccc aag ggc ctt agt tgt cac ttt cta tgc cac aaa aca gac aag aga   3079
Pro Lys Gly Leu Ser Cys His Phe Leu Cys His Lys Thr Asp Lys Arg
       1000                1005                1010

aag tcc ccc tgg gtc ctg tgg tgg aac att cgg aaa acc tgc tac caa   3127
Lys Ser Pro Trp Val Leu Trp Trp Asn Ile Arg Lys Thr Cys Tyr Gln
   1015                1020                1025

atc gtg aag cac agc tgg ttt gag agt ttc ata atc ttt gtt att ctg   3175
Ile Val Lys His Ser Trp Phe Glu Ser Phe Ile Ile Phe Val Ile Leu
1030                1035                1040                1045

ctg agc agt gga gcg ctg ata ttt gaa gat gtc aat ctc ccc agc cgg   3223
Leu Ser Ser Gly Ala Leu Ile Phe Glu Asp Val Asn Leu Pro Ser Arg
               1050                1055                1060

ccc caa gtt gag aaa tta cta agg tgt acc gat aat att ttc aca ttt   3271
Pro Gln Val Glu Lys Leu Leu Arg Cys Thr Asp Asn Ile Phe Thr Phe
           1065                1070                1075

att ttc ctc ctg gaa atg atc ctg aag tgg gtg gcc ttt gga ttc cgg   3319
Ile Phe Leu Leu Glu Met Ile Leu Lys Trp Val Ala Phe Gly Phe Arg
       1080                1085                1090

agg tat ttc acc agt gcc tgg tgc tgg ctt gat ttc ctc att gtg gtg   3367
Arg Tyr Phe Thr Ser Ala Trp Cys Trp Leu Asp Phe Leu Ile Val Val
   1095                1100                1105

gtg tct gtg ctc agt ctc atg aat cta cca agc ttg aag tcc ttc cgg   3415
Val Ser Val Leu Ser Leu Met Asn Leu Pro Ser Leu Lys Ser Phe Arg
1110                1115                1120                1125

act ctg cgg gcc ctg aga cct ctg cgg gcg ctg tcc cag ttt gaa gga   3463
Thr Leu Arg Ala Leu Arg Pro Leu Arg Ala Leu Ser Gln Phe Glu Gly
               1130                1135                1140

atg aag gtt gtc gtc tac gcc ctg atc agc gcc ata cct gcc att ctc   3511
Met Lys Val Val Val Tyr Ala Leu Ile Ser Ala Ile Pro Ala Ile Leu
           1145                1150                1155

aat gtc ttg ctg gtc tgc ctc att ttc tgg ctc gta ttt tgt atc ttg   3559
Asn Val Leu Leu Val Cys Leu Ile Phe Trp Leu Val Phe Cys Ile Leu
       1160                1165                1170

gga gta aat tta ttt tct ggg aag ttt gga agg tgc att aac ggg aca   3607
Gly Val Asn Leu Phe Ser Gly Lys Phe Gly Arg Cys Ile Asn Gly Thr
   1175                1180                1185
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gac ata aat atg tat ttg gat ttt acc gaa gtt ccg aac cga agc caa   3655
Asp Ile Asn Met Tyr Leu Asp Phe Thr Glu Val Pro Asn Arg Ser Gln
1190                1195                1200                1205

tgt aac att agt aat tac tcg tgg aag gtc ccg cag gtc aac ttt gac   3703
Cys Asn Ile Ser Asn Tyr Ser Trp Lys Val Pro Gln Val Asn Phe Asp
                1210                1215                1220

aac gtg ggg aat gcc tat ctc gcc ctg ctg caa gtg gca acc tat aag   3751
Asn Val Gly Asn Ala Tyr Leu Ala Leu Leu Gln Val Ala Thr Tyr Lys
           1225                1230                1235

ggc tgg ctg gaa atc atg aat gct gct gtc gat tcc aga gag aaa gac   3799
Gly Trp Leu Glu Ile Met Asn Ala Ala Val Asp Ser Arg Glu Lys Asp
      1240                1245                1250

gag cag ccg gac ttt gag gcg aac ctc tac gcg tat ctc tac ttt gtg   3847
Glu Gln Pro Asp Phe Glu Ala Asn Leu Tyr Ala Tyr Leu Tyr Phe Val
   1255                1260                1265

gtt ttt atc atc ttc ggc tcc ttc ttt acc ctg aac ctc ttt atc ggt   3895
Val Phe Ile Ile Phe Gly Ser Phe Phe Thr Leu Asn Leu Phe Ile Gly
1270                1275                1280                1285

gtt att att gac aac ttc aat cag cag cag aaa aag tta ggt ggc caa   3943
Val Ile Ile Asp Asn Phe Asn Gln Gln Gln Lys Lys Leu Gly Gly Gln
                1290                1295                1300

gac att ttt atg aca gaa gaa cag aag aaa tat tac aat gca atg aaa   3991
Asp Ile Phe Met Thr Glu Glu Gln Lys Lys Tyr Tyr Asn Ala Met Lys
           1305                1310                1315

aag tta gga acc aag aaa cct caa aag ccc atc cca agg ccc ctg aac   4039
Lys Leu Gly Thr Lys Lys Pro Gln Lys Pro Ile Pro Arg Pro Leu Asn
      1320                1325                1330

aan tgt caa gcc ttt gtg ttc gac ctg gtc aca agc cat gtc ttt gac   4087
Xaa Cys Gln Ala Phe Val Phe Asp Leu Val Thr Ser His Val Phe Asp
   1335                1340                1345

gtc atc att ctg ggt ctt att gtc tta aat atg att atc atg atg gct   4135
Val Ile Ile Leu Gly Leu Ile Val Leu Asn Met Ile Ile Met Met Ala
1350                1355                1360                1365

gaa tct gcc gac cag ccc aaa gat gtg aag aaa acc ttt gat atc ctc   4183
Glu Ser Ala Asp Gln Pro Lys Asp Val Lys Lys Thr Phe Asp Ile Leu
                1370                1375                1380

aac ata gcc ttc gtg gtc atc ttt acc ata gag tgt ctc atc aaa gtc   4231
Asn Ile Ala Phe Val Val Ile Phe Thr Ile Glu Cys Leu Ile Lys Val
           1385                1390                1395

ttt gct ttg agg caa cac tac ttc acc aat ggc tgg aac tta ttt gat   4279
Phe Ala Leu Arg Gln His Tyr Phe Thr Asn Gly Trp Asn Leu Phe Asp
      1400                1405                1410
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tgt gtg gtc gtg gtt ctt tct atc att agt acc ctg gtt tcc cgc ttg   4327
Cys Val Val Val Val Leu Ser Ile Ile Ser Thr Leu Val Ser Arg Leu
   1415                1420                1425

gag gac agt gac att tct ttc ccg ccc acg ctc ttc aga gtc gtc cgc   4375
Glu Asp Ser Asp Ile Ser Phe Pro Pro Thr Leu Phe Arg Val Val Arg
1430               1435                1440                1445

ttg gct cgg att ggt cga atc ctc agg ctg gtc cgg gct gcc cgg gga   4423
Leu Ala Arg Ile Gly Arg Ile Leu Arg Leu Val Arg Ala Ala Arg Gly
               1450                1455                1460

atc agg acc ctc ctc ttt gct ttg atg atg tct ctc ccc tct ctc ttc   4471
Ile Arg Thr Leu Leu Phe Ala Leu Met Met Ser Leu Pro Ser Leu Phe
          1465                1470                1475

aac atc ggt ctg ctg ctc ttc ctg gtg atg ttc att tac gcc atc ttt   4519
Asn Ile Gly Leu Leu Leu Phe Leu Val Met Phe Ile Tyr Ala Ile Phe
       1480                1485                1490

ggg atg agc tgg ttt tcc aaa gtg aag aag ggc tcc ggg atc gac gac   4567
Gly Met Ser Trp Phe Ser Lys Val Lys Lys Gly Ser Gly Ile Asp Asp
   1495                1500                1505

atc ttc aac ttc gag acc ttt acg ggc agc atg ctg tgc ctc ttc cag   4615
Ile Phe Asn Phe Glu Thr Phe Thr Gly Ser Met Leu Cys Leu Phe Gln
1510               1515                1520                1525

ata acc act tcg gct ggc tgg gat acc ctc ctc aac ccc atg ctg gag   4663
Ile Thr Thr Ser Ala Gly Trp Asp Thr Leu Leu Asn Pro Met Leu Glu
               1530                1535                1540

gca aaa gaa cac tgc aac tcc tcc tcc caa gac agc tgt cag cag ccg   4711
Ala Lys Glu His Cys Asn Ser Ser Ser Gln Asp Ser Cys Gln Gln Pro
          1545                1550                1555

cag ata gcc gtc gtc tac ttc gtc agt tac atc atc atc tcc ttc ctc   4759
Gln Ile Ala Val Val Tyr Phe Val Ser Tyr Ile Ile Ile Ser Phe Leu
       1560                1565                1570

atc gtg gtc aac atg tac atc gct gtg atc ctc gag aac ttc aac aca   4807
Ile Val Val Asn Met Tyr Ile Ala Val Ile Leu Glu Asn Phe Asn Thr
   1575                1580                1585

gcc acg gag gag agc gag gac cct ctg gga gag gac gac ttt gaa atc   4855
Ala Thr Glu Glu Ser Glu Asp Pro Leu Gly Glu Asp Asp Phe Glu Ile
1590               1595                1600                1605

ttc tat gag gtc tgg gag aag ttt gac ccc gag gcg tcg cag ttc atc   4903
Phe Tyr Glu Val Trp Glu Lys Phe Asp Pro Glu Ala Ser Gln Phe Ile
               1610                1615                1620

cag tat tcg gcc ctc tct gac ttt gcg gac gcc ctg ccg gag ccg ttg   4951
Gln Tyr Ser Ala Leu Ser Asp Phe Ala Asp Ala Leu Pro Glu Pro Leu
          1625                1630                1635
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cgt gtg gcc aag ccg aat aag ttt cag ttt cta gtg atg gac ttg ccc   4999
Arg Val Ala Lys Pro Asn Lys Phe Gln Phe Leu Val Met Asp Leu Pro
       1640                1645                1650

atg gtg atg ggc gac cgc ctc cat tgc atg gat gtt ctc ttt gct ttc   5047
Met Val Met Gly Asp Arg Leu His Cys Met Asp Val Leu Phe Ala Phe
   1655                1660                1665

act acc agg gtc ctc ggg gac tcc agc ggc ttg gat acc atg aaa acc   5095
Thr Thr Arg Val Leu Gly Asp Ser Ser Gly Leu Asp Thr Met Lys Thr
1670                1675                1680                1685

atg atg gag gag aag ttt atg gag gcc aac cct ttt aag aag ctc tac   5143
Met Met Glu Glu Lys Phe Met Glu Ala Asn Pro Phe Lys Lys Leu Tyr
               1690                1695                1700

gag ccc ata gtc acc acc acc aag agg aag gag gag gag caa ggc gcc   5191
Glu Pro Ile Val Thr Thr Thr Lys Arg Lys Glu Glu Glu Gln Gly Ala
          1705                1710                1715

gcc gtc atc cag agg gcc tac cgg aaa cac atg gag aag atg gtc aaa   5239
Ala Val Ile Gln Arg Ala Tyr Arg Lys His Met Glu Lys Met Val Lys
       1720                1725                1730

ctg agg ctg aag gac agg tca agt tca tcg cac cag gtg ttt tgc aat   5287
Leu Arg Leu Lys Asp Arg Ser Ser Ser Ser His Gln Val Phe Cys Asn
   1735                1740                1745

gga gac ttg tcc agc ttg gat gtg gcc aag gtc aag gtt cac aat gac   5335
Gly Asp Leu Ser Ser Leu Asp Val Ala Lys Val Lys Val His Asn Asp
1750                1755                1760                1765

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gcaggcggca gactcactga acacaggccg ttcgatctgt gtttttggct gaacgaggtg 5455

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Arg Pro Phe Thr Ser Asp Ser Leu Ala Ala Ile Glu Lys Arg Ile Ala
             20                  25                  30

Ile Gln Lys Glu Arg Lys Lys Ser Lys Asp Lys Ala Ala Ala Glu Pro
         35                  40                  45

Gln Pro Arg Pro Gln Leu Asp Leu Lys Ala Ser Arg Lys Leu Pro Lys
     50                  55                  60

Leu Tyr Gly Asp Ile Pro Pro Glu Leu Val Ala Lys Pro Leu Glu Asp
 65                  70                  75                  80

Leu Asp Pro Phe Tyr Lys Asp His Lys Thr Phe Met Val Leu Asn Lys
                 85                  90                  95

Lys Arg Thr Ile Tyr Arg Phe Ser Ala Lys Arg Ala Leu Phe Ile Leu
            100                 105                 110

Gly Pro Phe Asn Pro Leu Arg Ser Leu Met Ile Arg Ile Ser Val His
        115                 120                 125

Ser Val Phe Ser Met Phe Ile Ile Cys Thr Val Ile Ile Asn Cys Met
    130                 135                 140

Phe Met Ala Asn Ser Met Glu Arg Ser Phe Asp Asn Asp Ile Pro Glu
145                 150                 155                 160

Tyr Val Phe Ile Gly Ile Tyr Ile Leu Glu Ala Val Ile Lys Ile Leu
                165                 170                 175

Ala Arg Gly Phe Ile Val Asp Glu Phe Ser Phe Leu Arg Asp Pro Trp
            180                 185                 190

Asn Trp Leu Asp Phe Ile Val Ile Gly Thr Ala Ile Ala Thr Cys Phe
        195                 200                 205

Pro Gly Ser Gln Val Asn Leu Ser Ala Leu Arg Thr Phe Arg Val Phe
    210                 215                 220

Arg Ala Leu Lys Ala Ile Ser Val Ile Ser Gly Leu Lys Val Ile Val
225                 230                 235                 240

Gly Ala Leu Leu Arg Ser Val Lys Lys Leu Val Asp Val Met Val Leu
                245                 250                 255

Thr Leu Phe Cys Leu Ser Ile Phe Ala Leu Val Gly Gln Gln Leu Phe
            260                 265                 270

Met Gly Ile Leu Asn Gln Lys Cys Ile Lys His Asn Cys Gly Pro Asn
        275                 280                 285

Pro Ala Ser Asn Lys Asp Cys Phe Glu Lys Glu Lys Asp Ser Glu Asp
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    290                 295                 300

Phe Ile Met Cys Gly Thr Trp Leu Gly Ser Arg Pro Cys Pro Asn Gly
305                 310                 315                 320

Ser Thr Cys Asp Lys Thr Thr Leu Asn Pro Asp Asn Asn Tyr Thr Lys
                325                 330                 335

Phe Asp Asn Phe Gly Trp Ser Phe Leu Ala Met Phe Arg Val Met Thr
            340                 345                 350

Gln Asp Ser Trp Glu Arg Leu Tyr Arg Gln Ile Leu Arg Thr Ser Gly
        355                 360                 365

Ile Tyr Phe Val Phe Phe Phe Val Val Val Ile Phe Leu Gly Ser Phe
    370                 375                 380

Tyr Leu Leu Asn Leu Thr Leu Ala Val Val Thr Met Ala Tyr Glu Glu
385                 390                 395                 400

Gln Asn Arg Asn Val Ala Ala Glu Thr Glu Ala Lys Glu Lys Met Phe
                405                 410                 415

Gln Glu Ala Gln Gln Leu Leu Arg Glu Glu Lys Glu Ala Leu Val Ala
            420                 425                 430

Met Gly Ile Asp Arg Ser Ser Leu Asn Ser Leu Gln Ala Ser Ser Phe
        435                 440                 445

Ser Pro Lys Lys Arg Lys Phe Phe Gly Ser Lys Thr Arg Lys Ser Phe
    450                 455                 460

Phe Met Arg Gly Ser Lys Thr Ala Gln Ala Ser Ala Ser Asp Ser Glu
465                 470                 475                 480

Asp Asp Ala Ser Lys Asn Pro Gln Leu Leu Glu Gln Thr Lys Arg Leu
                485                 490                 495

Ser Gln Asn Leu Pro Val Asp Leu Phe Asp Glu His Val Asp Pro Leu
            500                 505                 510

His Arg Gln Arg Ala Leu Ser Ala Val Ser Ile Leu Thr Ile Thr Met
        515                 520                 525

Gln Glu Gln Glu Lys Phe Gln Glu Pro Cys Phe Pro Cys Gly Lys Asn
    530                 535                 540

Leu Ala Ser Lys Tyr Leu Val Trp Asp Cys Ser Pro Gln Trp Leu Cys
545                 550                 555                 560

Ile Lys Lys Val Leu Arg Thr Ile Met Thr Asp Pro Phe Thr Glu Leu
                565                 570                 575

Ala Ile Thr Ile Cys Ile Ile Ile Asn Thr Val Phe Leu Ala Val Glu
            580                 585                 590
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His His Asn Met Asp Asp Asn Leu Lys Thr Ile Leu Lys Ile Gly Asn
595 600 605

Trp Val Phe Thr Gly Ile Phe Ile Ala Glu Met Cys Leu Lys Ile Ile
610 615 620

Ala Leu Asp Pro Tyr His Tyr Phe Arg His Gly Trp Asn Val Phe Asp
625 630 635 640

Ser Ile Val Ala Leu Leu Ser Leu Ala Asp Val Xaa Tyr Asn Thr Leu
645 650 655

Ser Asp Asn Asn Arg Ser Phe Leu Ala Ser Leu Arg Val Leu Arg Val
660 665 670

Phe Lys Leu Ala Lys Ser Trp Pro Thr Leu Asn Thr Leu Ile Lys Ile
675 680 685

Ile Gly His Ser Val Gly Ala Leu Gly Asn Leu Thr Val Val Leu Thr
690 695 700

Ile Val Val Phe Ile Phe Ser Val Val Gly Met Arg Leu Phe Gly Thr
705 710 715 720

Lys Phe Asn Lys Thr Ala Tyr Ala Thr Gln Glu Arg Pro Arg Arg Arg
725 730 735

Trp His Met Asp Asn Phe Tyr His Ser Phe Leu Val Val Phe Arg Ile
740 745 750

Leu Cys Gly Glu Trp Ile Glu Asn Met Trp Gly Cys Met Gln Asp Met
755 760 765

Asp Gly Ser Pro Leu Cys Ile Ile Val Phe Val Leu Ile Met Val Ile
770 775 780

Gly Lys Leu Val Val Leu Asn Leu Phe Ile Ala Leu Leu Leu Asn Ser
785 790 795 800

Phe Ser Asn Glu Glu Lys Asp Gly Ser Leu Glu Gly Glu Thr Arg Lys
805 810 815

Thr Lys Val Gln Leu Ala Leu Asp Arg Phe Arg Arg Ala Phe Ser Phe
820 825 830

Met Leu His Ala Leu Gln Ser Phe Cys Cys Lys Lys Cys Arg Arg Lys
835 840 845

Asn Ser Pro Lys Pro Lys Glu Thr Thr Glu Ser Phe Ala Gly Glu Asn
850 855 860

Lys Asp Ser Ile Leu Pro Asp Ala Arg Pro Trp Lys Glu Tyr Asp Thr
865 870 875 880

Asp Met Ala Leu Tyr Thr Gly Gln Ala Gly Ala Pro Leu Ala Pro Leu
885 890 895

Ala Glu Val Glu Asp Asp Val Glu Tyr Cys Gly Glu Gly Gly Ala Leu
900 905 910

Pro Thr Ser Gln His Ser Ala Gly Val Gln Ala Gly Asp Leu Pro Pro
915 920 925

Glu Thr Lys Gln Leu Thr Ser Pro Asp Asp Gln Gly Val Glu Met Glu
930 935 940

Val Phe Ser Glu Glu Asp Leu His Leu Ser Ile Gln Ser Pro Arg Lys
945 950 955 960

Lys Ser Asp Ala Val Ser Met Leu Ser Glu Cys Ser Thr Ile Asp Leu
965 970 975

Asn Asp Ile Phe Arg Asn Leu Gln Lys Thr Val Ser Pro Lys Lys Gln
980 985 990

Pro Asp Arg Cys Phe Pro Lys Gly Leu Ser Cys His Phe Leu Cys His
995 1000 1005

Lys Thr Asp Lys Arg Lys Ser Pro Trp Val Leu Trp Trp Asn Ile Arg
1010 1015 1020

Lys Thr Cys Tyr Gln Ile Val Lys His Ser Trp Phe Glu Ser Phe Ile
1025 1030 1035 1040

Ile Phe Val Ile Leu Leu Ser Ser Gly Ala Leu Ile Phe Glu Asp Val
1045 1050 1055

Asn Leu Pro Ser Arg Pro Gln Val Glu Lys Leu Leu Arg Cys Thr Asp
1060 1065 1070

Asn Ile Phe Thr Phe Ile Phe Leu Leu Glu Met Ile Leu Lys Trp Val
1075 1080 1085

Ala Phe Gly Phe Arg Arg Tyr Phe Thr Ser Ala Trp Cys Trp Leu Asp
1090 1095 1100

Phe Leu Ile Val Val Val Ser Val Leu Ser Leu Met Asn Leu Pro Ser
1105 1110 1115 1120

Leu Lys Ser Phe Arg Thr Leu Arg Ala Leu Arg Pro Leu Arg Ala Leu
1125 1130 1135

Ser Gln Phe Glu Gly Met Lys Val Val Val Tyr Ala Leu Ile Ser Ala
1140 1145 1150

Ile Pro Ala Ile Leu Asn Val Leu Leu Val Cys Leu Ile Phe Trp Leu
1155 1160 1165

Val Phe Cys Ile Leu Gly Val Asn Leu Phe Ser Gly Lys Phe Gly Arg
1170 1175 1180

Cys Ile Asn Gly Thr Asp Ile Asn Met Tyr Leu Asp Phe Thr Glu Val
1185 1190 1195 1200

Pro Asn Arg Ser Gln Cys Asn Ile Ser Asn Tyr Ser Trp Lys Val Pro
1205 1210 1215

Gln Val Asn Phe Asp Asn Val Gly Asn Ala Tyr Leu Ala Leu Leu Gln
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Val Ala Thr Tyr Lys Gly Trp Leu Glu Ile Met Asn Ala Ala Val Asp
1235 1240 1245

Ser Arg Glu Lys Asp Glu Gln Pro Asp Phe Glu Ala Asn Leu Tyr Ala
1250 1255 1260

Tyr Leu Tyr Phe Val Val Phe Ile Ile Phe Gly Ser Phe Phe Thr Leu
1265 1270 1275 1280

Asn Leu Phe Ile Gly Val Ile Ile Asp Asn Phe Asn Gln Gln Gln Lys
1285 1290 1295

Lys Leu Gly Gly Gln Asp Ile Phe Met Thr Glu Glu Gln Lys Lys Tyr
1300 1305 1310

Tyr Asn Ala Met Lys Lys Leu Gly Thr Lys Lys Pro Gln Lys Pro Ile
1315 1320 1325

Pro Arg Pro Leu Asn Xaa Cys Gln Ala Phe Val Phe Asp Leu Val Thr
1330 1335 1340

Ser His Val Phe Asp Val Ile Ile Leu Gly Leu Ile Val Leu Asn Met
1345 1350 1355 1360

Ile Ile Met Met Ala Glu Ser Ala Asp Gln Pro Lys Asp Val Lys Lys
1365 1370 1375

Thr Phe Asp Ile Leu Asn Ile Ala Phe Val Val Ile Phe Thr Ile Glu
1380 1385 1390

Cys Leu Ile Lys Val Phe Ala Leu Arg Gln His Tyr Phe Thr Asn Gly
1395 1400 1405

Trp Asn Leu Phe Asp Cys Val Val Val Val Leu Ser Ile Ile Ser Thr
1410 1415 1420

Leu Val Ser Arg Leu Glu Asp Ser Asp Ile Ser Phe Pro Pro Thr Leu
1425 1430 1435 1440

Phe Arg Val Val Arg Leu Ala Arg Ile Gly Arg Ile Leu Arg Leu Val
1445 1450 1455

Arg Ala Ala Arg Gly Ile Arg Thr Leu Leu Phe Ala Leu Met Met Ser
1460 1465 1470

Leu Pro Ser Leu Phe Asn Ile Gly Leu Leu Leu Phe Leu Val Met Phe
1475 1480 1485

Ile Tyr Ala Ile Phe Gly Met Ser Trp Phe Ser Lys Val Lys Lys Gly
1490 1495 1500

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Ser Gly Ile Asp Asp Ile Phe Asn Phe Glu Thr Phe Thr Gly Ser Met
1505                1510                1515                1520

Leu Cys Leu Phe Gln Ile Thr Thr Ser Ala Gly Trp Asp Thr Leu Leu
                1525                1530                1535

Asn Pro Met Leu Glu Ala Lys Glu His Cys Asn Ser Ser Ser Gln Asp
            1540                1545                1550

Ser Cys Gln Gln Pro Gln Ile Ala Val Val Tyr Phe Val Ser Tyr Ile
        1555                1560                1565

Ile Ile Ser Phe Leu Ile Val Val Asn Met Tyr Ile Ala Val Ile Leu
    1570                1575                1580

Glu Asn Phe Asn Thr Ala Thr Glu Glu Ser Glu Asp Pro Leu Gly Glu
1585                1590                1595                1600

Asp Asp Phe Glu Ile Phe Tyr Glu Val Trp Glu Lys Phe Asp Pro Glu
                1605                1610                1615

Ala Ser Gln Phe Ile Gln Tyr Ser Ala Leu Ser Asp Phe Ala Asp Ala
            1620                1625                1630

Leu Pro Glu Pro Leu Arg Val Ala Lys Pro Asn Lys Phe Gln Phe Leu
        1635                1640                1645

Val Met Asp Leu Pro Met Val Met Gly Asp Arg Leu His Cys Met Asp
    1650                1655                1660

Val Leu Phe Ala Phe Thr Thr Arg Val Leu Gly Asp Ser Ser Gly Leu
1665                1670                1675                1680

Asp Thr Met Lys Thr Met Met Glu Glu Lys Phe Met Glu Ala Asn Pro
                1685                1690                1695

Phe Lys Lys Leu Tyr Glu Pro Ile Val Thr Thr Thr Lys Arg Lys Glu
            1700                1705                1710

Glu Glu Gln Gly Ala Ala Val Ile Gln Arg Ala Tyr Arg Lys His Met
        1715                1720                1725

Glu Lys Met Val Lys Leu Arg Leu Lys Asp Arg Ser Ser Ser Ser His
    1730                1735                1740

Gln Val Phe Cys Asn Gly Asp Leu Ser Ser Leu Asp Val Ala Lys Val
1745                1750                1755                1760

Lys Val His Asn Asp
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             20                  25                  30

Ile Gln Lys Glu Arg Lys Lys Ser Lys Asp Lys Ala Ala Ala Glu Pro
         35                  40                  45

Gln Pro Arg Pro Gln Leu Asp Leu Lys Ala Ser Arg Lys Leu Pro Lys
     50                  55                  60

Leu Tyr Gly Asp Ile Pro Pro Glu Leu Val Ala Lys Pro Leu Glu Asp
 65                  70                  75                  80

Leu Asp Pro Phe Tyr Lys Asp His Lys Thr Phe Met Val Leu Asn Lys
                 85                  90                  95

Lys Arg Thr Ile Tyr Arg Phe Ser Ala Lys Arg Ala Leu Phe Ile Leu
            100                 105                 110

Gly Pro Phe Asn Pro Leu Arg Ser Leu Met Ile Arg Ile Ser Val His
        115                 120                 125

Ser Val Phe Ser Met Phe Ile Ile Cys Thr Val Ile Ile Asn Cys Met
    130                 135                 140

Phe Met Ala Asn Ser Met Glu Arg Ser Phe Asp Asn Asp Ile Pro Glu
145                 150                 155                 160

Tyr Val Phe Ile Gly Ile Tyr Ile Leu Glu Ala Val Ile Lys Ile Leu
                165                 170                 175

Ala Arg Gly Phe Ile Val Asp Glu Phe Ser Phe Leu Arg Asp Pro Trp
            180                 185                 190

Asn Trp Leu Asp Phe Ile Val Ile Gly Thr Ala Ile Ala Thr Cys Phe
        195                 200                 205

Pro Gly Ser Gln Val Asn Leu Ser Ala Leu Arg Thr Phe Arg Val Phe
    210                 215                 220

Arg Ala Leu Lys Ala Ile Ser Val Ile Ser Gly Leu Lys Val Ile Val
225                 230                 235                 240

Gly Ala Leu Leu Arg Ser Val Lys Lys Leu Val Asp Val Met Val Leu
                245                 250                 255

Thr Leu Phe Cys Leu Ser Ile Phe Ala Leu Val Gly Gln Gln Leu Phe
            260                 265                 270
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Met Gly Ile Leu Asn Gln Lys Cys Ile Lys His Asn Cys Gly Pro Asn
        275                 280                 285

Pro Ala Ser Asn Lys Asp Cys Phe Glu Lys Glu Lys Asp Ser Glu Asp
    290                 295                 300

Phe Ile Met Cys Gly Thr Trp Leu Gly Ser Arg Pro Cys Pro Asn Gly
305                 310                 315                 320

Ser Thr Cys Asp Lys Thr Thr Leu Asn Pro Asp Asn Asn Tyr Thr Lys
                325                 330                 335

Phe Asp Asn Phe Gly Trp Ser Phe Leu Ala Met Phe Arg Val Met Thr
            340                 345                 350

Gln Asp Ser Trp Glu Arg Leu Tyr Arg Gln Ile Leu Arg Thr Ser Gly
        355                 360                 365

Ile Tyr Phe Val Phe Phe Phe Val Val Val Ile Phe Leu Gly Ser Phe
    370                 375                 380

Tyr Leu Leu Asn Leu Thr Leu Ala Val Val Thr Met Ala Tyr Glu Glu
385                 390                 395                 400

Gln Asn Arg Asn Val Ala Ala Glu Thr Glu Ala Lys Glu Lys Met Phe
                405                 410                 415

Gln Glu Ala Gln Gln Leu Leu Arg Glu Glu Lys Glu Ala Leu Val Ala
            420                 425                 430

Met Gly Ile Asp Arg Ser Ser Leu Asn Ser Leu Gln Ala Ser Ser Phe
        435                 440                 445

Ser Pro Lys Lys Arg Lys Phe Phe Gly Ser Lys Thr Arg Lys Ser Phe
    450                 455                 460

Phe Met Arg Gly Ser Lys Thr Ala Gln Ala Ser Ala Ser Asp Ser Glu
465                 470                 475                 480

Asp Asp Ala Ser Lys Asn Pro Gln Leu Leu Glu Gln Thr Lys Arg Leu
                485                 490                 495

Ser Gln Asn Leu Pro Val Asp Leu Phe Asp Glu His Val Asp Pro Leu
            500                 505                 510

His Arg Gln Arg Ala Leu Ser Ala Val Ser Ile Leu Thr Ile Thr Met
        515                 520                 525

Gln Glu Gln Glu Lys Phe Gln Glu Pro Cys Phe Pro Cys Gly Lys Asn
    530                 535                 540

Leu Ala Ser Lys Tyr Leu Val Trp Asp Cys Ser Pro Gln Trp Leu Cys
545                 550                 555                 560

Ile Lys Lys Val Leu Arg Thr Ile Met Thr Asp Pro Phe Thr Glu Leu
                565                 570                 575
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Ala Ile Thr Ile Cys Ile Ile Ile Asn Thr Val Phe Leu Ala Val Glu
            580                 585                 590

His His Asn Met Asp Asp Asn Leu Lys Thr Ile Leu Lys Ile Gly Asn
        595                 600                 605

Trp Val Phe Thr Gly Ile Phe Ile Ala Glu Met Cys Leu Lys Ile Ile
    610                 615                 620

Ala Leu Asp Pro Tyr His Tyr Phe Arg His Gly Trp Asn Val Phe Asp
625                 630                 635                 640

Ser Ile Val Ala Leu Leu Ser Leu Ala Asp Val Leu Tyr Asn Thr Leu
                645                 650                 655

Ser Asp Asn Asn Arg Ser Phe Leu Ala Ser Leu Arg Val Leu Arg Val
            660                 665                 670

Phe Lys Leu Ala Lys Ser Trp Pro Thr Leu Asn Thr Leu Ile Lys Ile
        675                 680                 685

Ile Gly His Ser Val Gly Ala Leu Gly Asn Leu Thr Val Val Leu Thr
    690                 695                 700

Ile Val Val Phe Ile Phe Ser Val Val Gly Met Arg Leu Phe Gly Thr
705                 710                 715                 720

Lys Phe Asn Lys Thr Ala Tyr Ala Thr Gln Glu Arg Pro Arg Arg Arg
                725                 730                 735

Trp His Met Asp Asn Phe Tyr His Ser Phe Leu Val Val Phe Arg Ile
            740                 745                 750

Leu Cys Gly Glu Trp Ile Glu Asn Met Trp Gly Cys Met Gln Asp Met
        755                 760                 765

Asp Gly Ser Pro Leu Cys Ile Ile Val Phe Val Leu Ile Met Val Ile
    770                 775                 780

Gly Lys Leu Val Val Leu Asn Leu Phe Ile Ala Leu Leu Leu Asn Ser
785                 790                 795                 800

Phe Ser Asn Glu Glu Lys Asp Gly Ser Leu Glu Gly Glu Thr Arg Lys
                805                 810                 815

Thr Lys Val Gln Leu Ala Leu Asp Arg Phe Arg Arg Ala Phe Ser Phe
            820                 825                 830

Met Leu His Ala Leu Gln Ser Phe Cys Cys Lys Lys Cys Arg Arg Lys
        835                 840                 845

Asn Ser Pro Lys Pro Lys Glu Thr Thr Glu Ser Phe Ala Gly Glu Asn
    850                 855                 860

Lys Asp Ser Ile Leu Pro Asp Ala Arg Pro Trp Lys Glu Tyr Asp Thr
865                 870                 875                 880
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Asp Met Ala Leu Tyr Thr Gly Gln Ala Gly Ala Pro Leu Ala Pro Leu
                885                 890                 895

Ala Glu Val Glu Asp Asp Val Glu Tyr Cys Gly Glu Gly Gly Ala Leu
            900                 905                 910

Pro Thr Ser Gln His Ser Ala Gly Val Gln Ala Gly Asp Leu Pro Pro
        915                 920                 925

Glu Thr Lys Gln Leu Thr Ser Pro Asp Asp Gln Gly Val Glu Met Glu
    930                 935                 940

Val Phe Ser Glu Glu Asp Leu His Leu Ser Ile Gln Ser Pro Arg Lys
945                 950                 955                 960

Lys Ser Asp Ala Val Ser Met Leu Ser Glu Cys Ser Thr Ile Asp Leu
                965                 970                 975

Asn Asp Ile Phe Arg Asn Leu Gln Lys Thr Val Ser Pro Lys Lys Gln
            980                 985                 990

Pro Asp Arg Cys Phe Pro Lys Gly Leu Ser Cys His Phe Leu Cys His
        995                1000                1005

Lys Thr Asp Lys Arg Lys Ser Pro Trp Val Leu Trp Trp Asn Ile Arg
   1010                1015                1020

Lys Thr Cys Tyr Gln Ile Val Lys His Ser Trp Phe Glu Ser Phe Ile
1025                1030                1035                1040

Ile Phe Val Ile Leu Leu Ser Ser Gly Ala Leu Ile Phe Glu Asp Val
                1045                1050                1055

Asn Leu Pro Ser Arg Pro Gln Val Glu Lys Leu Leu Arg Cys Thr Asp
            1060                1065                1070

Asn Ile Phe Thr Phe Ile Phe Leu Leu Glu Met Ile Leu Lys Trp Val
       1075                1080                1085

Ala Phe Gly Phe Arg Arg Tyr Phe Thr Ser Ala Trp Cys Trp Leu Asp
   1090                1095                1100

Phe Leu Ile Val Val Val Ser Val Leu Ser Leu Met Asn Leu Pro Ser
1105                1110                1115                1120

Leu Lys Ser Phe Arg Thr Leu Arg Ala Leu Arg Pro Leu Arg Ala Leu
                1125                1130                1135

Ser Gln Phe Glu Gly Met Lys Val Val Val Tyr Ala Leu Ile Ser Ala
            1140                1145                1150

Ile Pro Ala Ile Leu Asn Val Leu Leu Val Cys Leu Ile Phe Trp Leu
       1155                1160                1165

Val Phe Cys Ile Leu Gly Val Asn Leu Phe Ser Gly Lys Phe Gly Arg
   1170                1175                1180
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Cys Ile Asn Gly Thr Asp Ile Asn Met Tyr Leu Asp Phe Thr Glu Val
1185                1190                1195                1200

Pro Asn Arg Ser Gln Cys Asn Ile Ser Asn Tyr Ser Trp Lys Val Pro
                1205                1210                1215

Gln Val Asn Phe Asp Asn Val Gly Asn Ala Tyr Leu Ala Leu Leu Gln
            1220                1225                1230

Val Ala Thr Tyr Lys Gly Trp Leu Glu Ile Met Asn Ala Ala Val Asp
        1235                1240                1245

Ser Arg Glu Lys Asp Glu Gln Pro Asp Phe Glu Ala Asn Leu Tyr Ala
    1250                1255                1260

Tyr Leu Tyr Phe Val Val Phe Ile Ile Phe Gly Ser Phe Phe Thr Leu
1265                1270                1275                1280

Asn Leu Phe Ile Gly Val Ile Ile Asp Asn Phe Asn Gln Gln Gln Lys
                1285                1290                1295

Lys Leu Gly Gly Gln Asp Ile Phe Met Thr Glu Glu Gln Lys Lys Tyr
            1300                1305                1310

Tyr Asn Ala Met Lys Lys Leu Gly Thr Lys Lys Pro Gln Lys Pro Ile
        1315                1320                1325

Pro Arg Pro Leu Asn Arg Cys Gln Ala Phe Val Phe Asp Leu Val Thr
    1330                1335                1340

Ser His Val Phe Asp Val Ile Ile Leu Gly Leu Ile Val Leu Asn Met
1345                1350                1355                1360

Ile Ile Met Met Ala Glu Ser Ala Asp Gln Pro Lys Asp Val Lys Lys
                1365                1370                1375

Thr Phe Asp Ile Leu Asn Ile Ala Phe Val Val Ile Phe Thr Ile Glu
            1380                1385                1390

Cys Leu Ile Lys Val Phe Ala Leu Arg Gln His Tyr Phe Thr Asn Gly
        1395                1400                1405

Trp Asn Leu Phe Asp Cys Val Val Val Val Leu Ser Ile Ile Ser Thr
    1410                1415                1420

Leu Val Ser Arg Leu Glu Asp Ser Asp Ile Ser Phe Pro Pro Thr Leu
1425                1430                1435                1440

Phe Arg Val Val Arg Leu Ala Arg Ile Gly Arg Ile Leu Arg Leu Val
                1445                1450                1455

Arg Ala Ala Arg Gly Ile Arg Thr Leu Leu Phe Ala Leu Met Met Ser
            1460                1465                1470

Leu Pro Ser Leu Phe Asn Ile Gly Leu Leu Leu Phe Leu Val Met Phe
        1475                1480                1485
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Ile Tyr Ala Ile Phe Gly Met Ser Trp Phe Ser Lys Val Lys Lys Gly
   1490                1495                1500

Ser Gly Ile Asp Asp Ile Phe Asn Phe Glu Thr Phe Thr Gly Ser Met
1505                1510                1515                1520

Leu Cys Leu Phe Gln Ile Thr Thr Ser Ala Gly Trp Asp Thr Leu Leu
                1525                1530                1535

Asn Pro Met Leu Glu Ala Lys Glu His Cys Asn Ser Ser Ser Gln Asp
            1540                1545                1550

Ser Cys Gln Gln Pro Gln Ile Ala Val Val Tyr Phe Val Ser Tyr Ile
        1555                1560                1565

Ile Ile Ser Phe Leu Ile Val Val Asn Met Tyr Ile Ala Val Ile Leu
   1570                1575                1580

Glu Asn Phe Asn Thr Ala Thr Glu Glu Ser Glu Asp Pro Leu Gly Glu
1585                1590                1595                1600

Asp Asp Phe Glu Ile Phe Tyr Glu Val Trp Glu Lys Phe Asp Pro Glu
                1605                1610                1615

Ala Ser Gln Phe Ile Gln Tyr Ser Ala Leu Ser Asp Phe Ala Asp Ala
            1620                1625                1630

Leu Pro Glu Pro Leu Arg Val Ala Lys Pro Asn Lys Phe Gln Phe Leu
        1635                1640                1645

Val Met Asp Leu Pro Met Val Met Gly Asp Arg Leu His Cys Met Asp
   1650                1655                1660

Val Leu Phe Ala Phe Thr Thr Arg Val Leu Gly Asp Ser Ser Gly Leu
1665                1670                1675                1680

Asp Thr Met Lys Thr Met Met Glu Glu Lys Phe Met Glu Ala Asn Pro
                1685                1690                1695

Phe Lys Lys Leu Tyr Glu Pro Ile Val Thr Thr Thr Lys Arg Lys Glu
            1700                1705                1710

Glu Glu Gln Gly Ala Ala Val Ile Gln Arg Ala Tyr Arg Lys His Met
        1715                1720                1725

Glu Lys Met Val Lys Leu Arg Leu Lys Asp Arg Ser Ser Ser Ser His
   1730                1735                1740

Gln Val Phe Cys Asn Gly Asp Leu Ser Ser Leu Asp Val Ala Lys Val
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Lys Val His Asn Asp
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Asp Glu Arg Asn Phe Arg Pro Phe Thr Phe Asp Ser Leu Ala Ala Ile
15 20 25

gag aag cgg atc acc atc caa aag gag aag aag aaa tcc aaa gac aag 147
Glu Lys Arg Ile Thr Ile Gln Lys Glu Lys Lys Lys Ser Lys Asp Lys
30 35 40

gca gca act gag ccc cag cct cgg cct cag ctc gac cta aag gcc tcc 195
Ala Ala Thr Glu Pro Gln Pro Arg Pro Gln Leu Asp Leu Lys Ala Ser
45 50 55

agg aag tta cct aag ctc tat ggc gac gtt ccc cct gac ctt ata gcg 243
Arg Lys Leu Pro Lys Leu Tyr Gly Asp Val Pro Pro Asp Leu Ile Ala
60 65 70 75

aag ccc ctg gaa gat ctg gac cca ttt tac aaa gac cat aag aca ttc 291
Lys Pro Leu Glu Asp Leu Asp Pro Phe Tyr Lys Asp His Lys Thr Phe
80 85 90

atg gta ttg aac aag aag aga aca atc tat cgc ttc agc gcc aag agg 339
Met Val Leu Asn Lys Lys Arg Thr Ile Tyr Arg Phe Ser Ala Lys Arg
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gcc ttg ttc att ctg ggg cct ttt aat ccc atc aga agc ttc atg att 387
Ala Leu Phe Ile Leu Gly Pro Phe Asn Pro Ile Arg Ser Phe Met Ile
110 115 120

cgc atc tct gtc cat tca gtc ttc agc atg ttc att atc tgc aca gtg 435
Arg Ile Ser Val His Ser Val Phe Ser Met Phe Ile Ile Cys Thr Val
125 130 135

atc atc aac tgt atg ttc atg gct aat aat tct tct gtg gac agt cgt 483
Ile Ile Asn Cys Met Phe Met Ala Asn Asn Ser Ser Val Asp Ser Arg
140 145 150 155

cct agc agt aac att ccc gaa tac gtc ttc att ggg att tat gtt tta 531
Pro Ser Ser Asn Ile Pro Glu Tyr Val Phe Ile Gly Ile Tyr Val Leu

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                160                 165                 170

gaa gct gtg att aaa ata ttg gca aga ggc ttc att gtg gat gag ttt   579
Glu Ala Val Ile Lys Ile Leu Ala Arg Gly Phe Ile Val Asp Glu Phe
            175                 180                 185

tcc tac ctc cga gat cct tgg aac tgg ctg gac ttc att gtc atc gga   627
Ser Tyr Leu Arg Asp Pro Trp Asn Trp Leu Asp Phe Ile Val Ile Gly
        190                 195                 200

aca gcg ata gcg cct tgt ttt ctc ggt aac aaa gtc aat aat ctt tcc   675
Thr Ala Ile Ala Pro Cys Phe Leu Gly Asn Lys Val Asn Asn Leu Ser
    205                 210                 215

act cta cgt acc ttc cga gtg ttg aga gct ctg aaa gcc att tct gta   723
Thr Leu Arg Thr Phe Arg Val Leu Arg Ala Leu Lys Ala Ile Ser Val
220                 225                 230                 235

atc tca ggt ctg aag gtc atc gtg ggt gcc ctg ctg cgc tcc gtg aag   771
Ile Ser Gly Leu Lys Val Ile Val Gly Ala Leu Leu Arg Ser Val Lys
                240                 245                 250

aag cta gtg gac gtg atg gtc ctc act ctc ttt tgc ctc agc atc ttt   819
Lys Leu Val Asp Val Met Val Leu Thr Leu Phe Cys Leu Ser Ile Phe
            255                 260                 265

gcc ctg gtt ggt cag cag ctc ttc atg gga att ctg agc cag aaa tgt   867
Ala Leu Val Gly Gln Gln Leu Phe Met Gly Ile Leu Ser Gln Lys Cys
        270                 275                 280

att aag gac gac tgt ggc cct aac gct ttt tcc aac aag gat tgc ttt   915
Ile Lys Asp Asp Cys Gly Pro Asn Ala Phe Ser Asn Lys Asp Cys Phe
    285                 290                 295

gta aaa gaa aat gat agc gag gac ttc ata atg tgt ggc aac tgg ctc   963
Val Lys Glu Asn Asp Ser Glu Asp Phe Ile Met Cys Gly Asn Trp Leu
300                 305                 310                 315

ggc aga aga tcc tgc ccc gat ggt tcc acg tgc aat aaa acc aca ttt   1011
Gly Arg Arg Ser Cys Pro Asp Gly Ser Thr Cys Asn Lys Thr Thr Phe
                320                 325                 330

aac cca gat tat aat tat aca aac ttt gac agc ttt ggc tgg tct ttt   1059
Asn Pro Asp Tyr Asn Tyr Thr Asn Phe Asp Ser Phe Gly Trp Ser Phe
            335                 340                 345

ctc gcc atg ttc cgg gtt atg act caa gac tcc tgg gag aag ctt tat   1107
Leu Ala Met Phe Arg Val Met Thr Gln Asp Ser Trp Glu Lys Leu Tyr
        350                 355                 360

cga cag atc ctt cgc acc tcc ggg atc tac ttt gtc ttc ttc ttc gtg   1155
Arg Gln Ile Leu Arg Thr Ser Gly Ile Tyr Phe Val Phe Phe Phe Val
    365                 370                 375

gtc gtc atc ttc ctg ggc tct ttc tac ctg ctt aac tta acc ctg gct   1203
Val Val Ile Phe Leu Gly Ser Phe Tyr Leu Leu Asn Leu Thr Leu Ala
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380                 385                 390                 395

gtc gtc acc atg gct tac gag gaa cag aac aga aat gtc gct gcc gag   1251
Val Val Thr Met Ala Tyr Glu Glu Gln Asn Arg Asn Val Ala Ala Glu
                400                 405                 410

aca gag gcc aag gag aag atg ttt cag gaa gcc cag cag ctg ttg agg   1299
Thr Glu Ala Lys Glu Lys Met Phe Gln Glu Ala Gln Gln Leu Leu Arg
            415                 420                 425

gag gaa aag gag gct ctg gtt gcc atg gga att gac aga act tcc ctt   1347
Glu Glu Lys Glu Ala Leu Val Ala Met Gly Ile Asp Arg Thr Ser Leu
        430                 435                 440

aat tcc ctc caa gct tcg tcc ttt tcc cca aag aag agg aag ttt ttt   1395
Asn Ser Leu Gln Ala Ser Ser Phe Ser Pro Lys Lys Arg Lys Phe Phe
    445                 450                 455

ggc agt aag aca aga aag tcc ttc ttt atg aga ggg tcc aag aca gcc   1443
Gly Ser Lys Thr Arg Lys Ser Phe Phe Met Arg Gly Ser Lys Thr Ala
460                 465                 470                 475

cga gcc tca gcg tcc gat tca gag gac gat gcc tct aaa aac cca caa   1491
Arg Ala Ser Ala Ser Asp Ser Glu Asp Asp Ala Ser Lys Asn Pro Gln
                480                 485                 490

ctc ctt gag caa aca aaa cga cta tcc cag aac ttg ccc gta gaa ctc   1539
Leu Leu Glu Gln Thr Lys Arg Leu Ser Gln Asn Leu Pro Val Glu Leu
            495                 500                 505

ttt gat gag cac gtg gac ccc ctc cat agg cag aga gcg ctg agt gcc   1587
Phe Asp Glu His Val Asp Pro Leu His Arg Gln Arg Ala Leu Ser Ala
        510                 515                 520

gtc agt atc tta acc atc acc atg cag gaa caa gaa aaa tcc cag gag   1635
Val Ser Ile Leu Thr Ile Thr Met Gln Glu Gln Glu Lys Ser Gln Glu
    525                 530                 535

cct tgt ttc ccg tgt ggg aaa aac ttg gca tcc aag tac ctg gtg tgg   1683
Pro Cys Phe Pro Cys Gly Lys Asn Leu Ala Ser Lys Tyr Leu Val Trp
540                 545                 550                 555

gaa tgt agc cct ccg tgg ctg tgc ata aag aag gtc ctg cag act atc   1731
Glu Cys Ser Pro Pro Trp Leu Cys Ile Lys Lys Val Leu Gln Thr Ile
                560                 565                 570

atg aca gac ccc ttc act gag ctg gcc atc acc atc tgc atc atc gtc   1779
Met Thr Asp Pro Phe Thr Glu Leu Ala Ile Thr Ile Cys Ile Ile Val
            575                 580                 585

aat act gtc ttc ttg gcc atg gaa cac cac aat atg gat aac tct tta   1827
Asn Thr Val Phe Leu Ala Met Glu His His Asn Met Asp Asn Ser Leu
        590                 595                 600

aaa gac ata ctg aaa ata gga aac tgg gtt ttc act gga att ttc ata   1875
Lys Asp Ile Leu Lys Ile Gly Asn Trp Val Phe Thr Gly Ile Phe Ile
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    605                 610                 615

gcg gaa atg tgt ctc aag atc att gcg cta gac cct tac cac tac ttc   1923
Ala Glu Met Cys Leu Lys Ile Ile Ala Leu Asp Pro Tyr His Tyr Phe
620                 625                 630                 635

cgg cac ggc tgg aac atc ttt gac agc att gtg gcc ctt gtg agt ctc   1971
Arg His Gly Trp Asn Ile Phe Asp Ser Ile Val Ala Leu Val Ser Leu
                640                 645                 650

gct gac gtg ctc ttc cac aaa ctg tct aaa aac ctc tcc ttc ttg gct   2019
Ala Asp Val Leu Phe His Lys Leu Ser Lys Asn Leu Ser Phe Leu Ala
            655                 660                 665

tcc ctc aga gtg ctg agg gtc ttc aag tta gcc aaa tcc tgg ccc aca   2067
Ser Leu Arg Val Leu Arg Val Phe Lys Leu Ala Lys Ser Trp Pro Thr
        670                 675                 680

tta aac act ctc att aag atc atc ggc cac tcc gtg ggt gcg ctc gga   2115
Leu Asn Thr Leu Ile Lys Ile Ile Gly His Ser Val Gly Ala Leu Gly
    685                 690                 695

aac ctg act gtg gtc cta acg atc gtg gtc ttc atc ttt tcc gtg gtt   2163
Asn Leu Thr Val Val Leu Thr Ile Val Val Phe Ile Phe Ser Val Val
700                 705                 710                 715

ggc atg cgg ctc ttt ggt gcc aag ttt aac aag act tgc tcc acc tct   2211
Gly Met Arg Leu Phe Gly Ala Lys Phe Asn Lys Thr Cys Ser Thr Ser
                720                 725                 730

ccg gag tcc ctc cgg cgc tgg cac atg ggt gat ttc tac cat tcc ttc   2259
Pro Glu Ser Leu Arg Arg Trp His Met Gly Asp Phe Tyr His Ser Phe
            735                 740                 745

ctg gtg gtg ttc cgc atc ctc tgt ggg gag tgg atc gag aac atg tgg   2307
Leu Val Val Phe Arg Ile Leu Cys Gly Glu Trp Ile Glu Asn Met Trp
        750                 755                 760

gaa tgc atg cag gag atg gaa ggc tcc ccg ctg tgt gtc atc gtc ttt   2355
Glu Cys Met Gln Glu Met Glu Gly Ser Pro Leu Cys Val Ile Val Phe
    765                 770                 775

gtg ctg atc atg gtg gtc ggg aag ctc gtg gtg ctt aac ctc ttc att   2403
Val Leu Ile Met Val Val Gly Lys Leu Val Val Leu Asn Leu Phe Ile
780                 785                 790                 795

gcc ttg ctg ctc aat tcc ttc agc aat gag gaa aag gat ggg aac cca   2451
Ala Leu Leu Leu Asn Ser Phe Ser Asn Glu Glu Lys Asp Gly Asn Pro
                800                 805                 810

gaa gga gag acc agg aaa acc aaa gtg cag cta gcc ctg gat cgg ttc   2499
Glu Gly Glu Thr Arg Lys Thr Lys Val Gln Leu Ala Leu Asp Arg Phe
            815                 820                 825

agc cga gcg ttc tac ttc atg gcg cgc gct ctt cag aat ttc tgt tgc   2547
Ser Arg Ala Phe Tyr Phe Met Ala Arg Ala Leu Gln Asn Phe Cys Cys
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        830                 835                 840

aag aga tgc agg agg caa aac tcg cca aag cca aat gag gca aca gaa   2595
Lys Arg Cys Arg Arg Gln Asn Ser Pro Lys Pro Asn Glu Ala Thr Glu
    845                 850                 855

agc ttt gct ggt gag agt aga gac aca gcc acc ctg gat aca agg tcc   2643
Ser Phe Ala Gly Glu Ser Arg Asp Thr Ala Thr Leu Asp Thr Arg Ser
860                 865                 870                 875

tgg aag gag tat gat tca gaa atg act ctg tac act ggg cag gcc ggg   2691
Trp Lys Glu Tyr Asp Ser Glu Met Thr Leu Tyr Thr Gly Gln Ala Gly
                880                 885                 890

gct cca ctg gcc cca ctg gca aaa gaa gag gac gat atg gaa tgt tgt   2739
Ala Pro Leu Ala Pro Leu Ala Lys Glu Glu Asp Asp Met Glu Cys Cys
            895                 900                 905

ggt gaa tgt gat gcc tca cct acc tca cag cct agt gag gaa gct cag   2787
Gly Glu Cys Asp Ala Ser Pro Thr Ser Gln Pro Ser Glu Glu Ala Gln
        910                 915                 920

gcc tgt gac ctc cct ctg aag acc aag cgg ctc ccc agc cca gat gac   2835
Ala Cys Asp Leu Pro Leu Lys Thr Lys Arg Leu Pro Ser Pro Asp Asp
    925                 930                 935

cac ggg gtt gaa atg gaa gtg ttt tcc gaa gaa gat ccg aat tta acc   2883
His Gly Val Glu Met Glu Val Phe Ser Glu Glu Asp Pro Asn Leu Thr
940                 945                 950                 955

ata cag agt gct cga aag aag tct gat gcg gca agc atg ctc tca gaa   2931
Ile Gln Ser Ala Arg Lys Lys Ser Asp Ala Ala Ser Met Leu Ser Glu
                960                 965                 970

tgc agc aca ata gac ctg aat gat atc ttt aga aat tta cag aaa aca   2979
Cys Ser Thr Ile Asp Leu Asn Asp Ile Phe Arg Asn Leu Gln Lys Thr
            975                 980                 985

gtt tcc ccc caa aag caa cca gat cga tgc ttt ccc aag ggc ctc agt   3027
Val Ser Pro Gln Lys Gln Pro Asp Arg Cys Phe Pro Lys Gly Leu Ser
        990                 995                1000

tgt atc ttt cta tgt tgc aaa aca atc aaa aaa aag tcc ccc tgg gtc   3075
Cys Ile Phe Leu Cys Cys Lys Thr Ile Lys Lys Lys Ser Pro Trp Val
   1005                1010                1015

ctg tgg tgg aat ctt cgg aaa acc tgc tac caa atc gtg aag cat agc   3123
Leu Trp Trp Asn Leu Arg Lys Thr Cys Tyr Gln Ile Val Lys His Ser
1020                1025                1030                1035

tgg ttt gag agc ttc ata att ttt gtc atc ctg ctg agc agc gga gca   3171
Trp Phe Glu Ser Phe Ile Ile Phe Val Ile Leu Leu Ser Ser Gly Ala
                1040                1045                1050

ctg ata ttc gaa gat gtc aat ctt ccc agc cgg ccc caa gtt gaa aaa   3219
Leu Ile Phe Glu Asp Val Asn Leu Pro Ser Arg Pro Gln Val Glu Lys
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          1055                1060                1065

tta ctg aag tgt acc gat aat att ttc aca ttt att ttt ctc ctg gaa   3267
Leu Leu Lys Cys Thr Asp Asn Ile Phe Thr Phe Ile Phe Leu Leu Glu
       1070                1075                1080

atg att ttg aag tgg gtg gcc ttt gga ttc cgg aag tat ttc acc agt   3315
Met Ile Leu Lys Trp Val Ala Phe Gly Phe Arg Lys Tyr Phe Thr Ser
   1085                1090                1095

gcc tgg tgc tgg ctc gat ttc ctc att gtg gtg gtg tct gtg ctc agc   3363
Ala Trp Cys Trp Leu Asp Phe Leu Ile Val Val Val Ser Val Leu Ser
1100                1105                1110                1115

ctc acg aac tta cca aac ttg aag tcc ttc cgg aat ctg cga gcg ctg   3411
Leu Thr Asn Leu Pro Asn Leu Lys Ser Phe Arg Asn Leu Arg Ala Leu
               1120                1125                1130

aga cct ctg cgg gca ctg tct cag ttt gaa gga atg aag gtt gtt gtc   3459
Arg Pro Leu Arg Ala Leu Ser Gln Phe Glu Gly Met Lys Val Val Val
          1135                1140                1145

aat gcc ctc atg agt gcc ata cct gcc atc ctc aat gtc ttg ctg gtc   3507
Asn Ala Leu Met Ser Ala Ile Pro Ala Ile Leu Asn Val Leu Leu Val
       1150                1155                1160

tgc ctc att ttc tgg ctc ata ttt tgt atc ctg gga gta aat ttt ttt   3555
Cys Leu Ile Phe Trp Leu Ile Phe Cys Ile Leu Gly Val Asn Phe Phe
   1165                1170                1175

tct ggg aag ttt gga aga tgc att aat gga aca gac ata aat aaa tat   3603
Ser Gly Lys Phe Gly Arg Cys Ile Asn Gly Thr Asp Ile Asn Lys Tyr
1180                1185                1190                1195

ttc aac gct tcc aat gtt cca aac caa agc caa tgt tta gtt agt aat   3651
Phe Asn Ala Ser Asn Val Pro Asn Gln Ser Gln Cys Leu Val Ser Asn
               1200                1205                1210

tac acg tgg aaa gtc ccg aat gtc aac ttt gac aac gtg ggg aat gcc   3699
Tyr Thr Trp Lys Val Pro Asn Val Asn Phe Asp Asn Val Gly Asn Ala
          1215                1220                1225

tac ctt gcc ctg ctg caa gtg gcg acc tat aag ggc tgg ctg gac att   3747
Tyr Leu Ala Leu Leu Gln Val Ala Thr Tyr Lys Gly Trp Leu Asp Ile
       1230                1235                1240

atg aat gca gct gtt gat tcc aga ggg aaa gat gag cag ccg gcc ttt   3795
Met Asn Ala Ala Val Asp Ser Arg Gly Lys Asp Glu Gln Pro Ala Phe
   1245                1250                1255

gag gcg aat cta tac gca tac ctt tac ttc gtg gtt ttt atc atc ttc   3843
Glu Ala Asn Leu Tyr Ala Tyr Leu Tyr Phe Val Val Phe Ile Ile Phe
1260                1265                1270                1275

ggc tca ttc ttt acc ctg aac ctc ttt atc ggt gtt att att gac aac   3891
Gly Ser Phe Phe Thr Leu Asn Leu Phe Ile Gly Val Ile Ile Asp Asn
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                1280                1285                1290

ttc aat cag cag cag aaa aag tta ggt ggc caa gac att ttt atg aca   3939
Phe Asn Gln Gln Gln Lys Lys Leu Gly Gly Gln Asp Ile Phe Met Thr
            1295                1300                1305

gaa gaa cag aag aaa tat tac aat gca atg aaa aag tta gga acc aag   3987
Glu Glu Gln Lys Lys Tyr Tyr Asn Ala Met Lys Lys Leu Gly Thr Lys
        1310                1315                1320

aag cct caa aag ccc atc cca agg ccc ctg aac aaa tgt caa gcc ttc   4035
Lys Pro Gln Lys Pro Ile Pro Arg Pro Leu Asn Lys Cys Gln Ala Phe
    1325                1330                1335

gtg ttc gat ttg gtc aca agc cag gtc ttt gac gtc atc att ctg ggt   4083
Val Phe Asp Leu Val Thr Ser Gln Val Phe Asp Val Ile Ile Leu Gly
1340                1345                1350                1355

ctt att gtc aca aac atg att atc atg atg gct gaa tct gaa ggc cag   4131
Leu Ile Val Thr Asn Met Ile Ile Met Met Ala Glu Ser Glu Gly Gln
                1360                1365                1370

ccc aac gaa gtg aag aaa atc ttt gat att ctc aac ata gtc ttc gtg   4179
Pro Asn Glu Val Lys Lys Ile Phe Asp Ile Leu Asn Ile Val Phe Val
            1375                1380                1385

gtc atc ttt acc gta gag tgt ctc atc aaa gtc ttt gct ttg agg caa   4227
Val Ile Phe Thr Val Glu Cys Leu Ile Lys Val Phe Ala Leu Arg Gln
        1390                1395                1400

cac tac ttc acc aat ggc tgg aac tta ttt gat tgt gtg gtc gtg gtt   4275
His Tyr Phe Thr Asn Gly Trp Asn Leu Phe Asp Cys Val Val Val Val
    1405                1410                1415

ctt tcc atc att agt acc ttg gtt tct ggc ttg gag aac agc aac gtc   4323
Leu Ser Ile Ile Ser Thr Leu Val Ser Gly Leu Glu Asn Ser Asn Val
1420                1425                1430                1435

ttc ccg ccc aca ctc ttc agg att gtc cgc ttg gct cgg atc ggt cga   4371
Phe Pro Pro Thr Leu Phe Arg Ile Val Arg Leu Ala Arg Ile Gly Arg
                1440                1445                1450

atc ctc aga ctg gtc cgg gcg gct cga gga atc agg aca ctc ctt ttc   4419
Ile Leu Arg Leu Val Arg Ala Ala Arg Gly Ile Arg Thr Leu Leu Phe
            1455                1460                1465

gcg ttg atg atg tct ctc ccc tct ctc ttc aac att ggt ctg ctt ctc   4467
Ala Leu Met Met Ser Leu Pro Ser Leu Phe Asn Ile Gly Leu Leu Leu
        1470                1475                1480

ttt ctg gtg atg ttc att tat gcc atc ttt ggg atg aac tgg ttt tcc   4515
Phe Leu Val Met Phe Ile Tyr Ala Ile Phe Gly Met Asn Trp Phe Ser
    1485                1490                1495

aaa gtg aag aga ggc tct ggg att gat gac atc ttc aac ttt gac act   4563
Lys Val Lys Arg Gly Ser Gly Ile Asp Asp Ile Phe Asn Phe Asp Thr
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1500                1505                1510                1515

ttc tcg ggc agc atg ctc tgc ctc ttc cag ata acc act tca gcc ggc   4611
Phe Ser Gly Ser Met Leu Cys Leu Phe Gln Ile Thr Thr Ser Ala Gly
                1520                1525                1530

tgg gat gct ctc ctc aac ccc atg ctg gaa tca aaa gcc tct tgc aat   4659
Trp Asp Ala Leu Leu Asn Pro Met Leu Glu Ser Lys Ala Ser Cys Asn
            1535                1540                1545

tcc tcc tcc caa gag agc tgt cag cag ccg cag ata gcc ata gtc tac   4707
Ser Ser Ser Gln Glu Ser Cys Gln Gln Pro Gln Ile Ala Ile Val Tyr
        1550                1555                1560

ttc gtc agc tac atc atc atc tcc ttt ctc att gtg gtt aac atg tac   4755
Phe Val Ser Tyr Ile Ile Ile Ser Phe Leu Ile Val Val Asn Met Tyr
    1565                1570                1575

ata gct gtg att cta gag aac ttc aac aca gcc aca gag gag agc gag   4803
Ile Ala Val Ile Leu Glu Asn Phe Asn Thr Ala Thr Glu Glu Ser Glu
1580                1585                1590                1595

gac ccc ctg ggc gaa gac gac ttt gag atc ttc tat gag atc tgg gag   4851
Asp Pro Leu Gly Glu Asp Asp Phe Glu Ile Phe Tyr Glu Ile Trp Glu
                1600                1605                1610

aag ttt gac ccc gaa gca aca cag ttc atc cag tac tca tcc ctc tct   4899
Lys Phe Asp Pro Glu Ala Thr Gln Phe Ile Gln Tyr Ser Ser Leu Ser
            1615                1620                1625

gac ttc gcc gac gcc ctg ccc gag ccg ttg cgt gtg gcc aag ccc aac   4947
Asp Phe Ala Asp Ala Leu Pro Glu Pro Leu Arg Val Ala Lys Pro Asn
        1630                1635                1640

agg ttt cag ttt ctc atg atg gac ttg ccc atg gtg atg ggt gat cgc   4995
Arg Phe Gln Phe Leu Met Met Asp Leu Pro Met Val Met Gly Asp Arg
    1645                1650                1655

ctc cat tgc atg gat gtt ctc ttt gct ttc acc acc agg gtc ctc ggg   5043
Leu His Cys Met Asp Val Leu Phe Ala Phe Thr Thr Arg Val Leu Gly
1660                1665                1670                1675

aac tcc agc ggc ttg gat acc atg aaa gcc atg atg gag gag aag ttc   5091
Asn Ser Ser Gly Leu Asp Thr Met Lys Ala Met Met Glu Glu Lys Phe
                1680                1685                1690

atg gag gcc aat cct ttc aag aag ttg tac gag ccc att gtc acc acc   5139
Met Glu Ala Asn Pro Phe Lys Lys Leu Tyr Glu Pro Ile Val Thr Thr
            1695                1700                1705

aca aag agg aag gag gag gag gaa tgt gcc gct gtc atc cag agg gcc   5187
Thr Lys Arg Lys Glu Glu Glu Glu Cys Ala Ala Val Ile Gln Arg Ala
        1710                1715                1720

tac cgg aga cac atg gag aag atg atc aag ctg aag ctg aaa ggc agg   5235
Tyr Arg Arg His Met Glu Lys Met Ile Lys Leu Lys Leu Lys Gly Arg
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   1725                1730                1735

tca agt tca tcg ctc cag gtg ttt tgc aat gga gac ttg tct agc ttg   5283
Ser Ser Ser Ser Leu Gln Val Phe Cys Asn Gly Asp Leu Ser Ser Leu
1740               1745                1750                1755

gat gtg ccc aag atc aag gtt cat tgt gac tgaaaccccc acctgcacgc     5333
Asp Val Pro Lys Ile Lys Val His Cys Asp
                1760                1765

ctacctcaca gcctcacagc tcagccccca gcctctggcg aacaagcggc ggactcaccg 5393

aacaggccgt tcaacttgtt tttttgggtg aaagaggtga taggttggtg tccattttta 5453

aatgattctt ggaaagattg aacgtcggaa catgttagaa aggactgcca aggacatcca 5513

cagtaacgga aggcctgaag gacagttcaa attatgtaaa gaaacgagaa ggaaaggtca 5573

catgtctgtt cagttttaag tatgtgacct gccacatgta gctcctttgc atgttaagtg 5633

agaagtcaaa accctgccat aagtaaatag ctttgttgca ggtgtttcta ccagtgctgc 5693

ggatttgggt gtatggctca aacctgaaag catgactctg acttgtcagc accccaactt 5753

tcagaagctc tgatctctgt cctaggtgtt tgacaaataa atacataaaa naaaaaaaaa 5813

aaaaaaaaa                                                        5822
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<210> 5
<211> 1765
<212> PRT
<213> Mus musculus

<400> 5

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Met Glu Glu Arg Tyr Tyr Pro Val Ile Phe Pro Asp Glu Arg Asn Phe
  1               5                  10                  15

Arg Pro Phe Thr Phe Asp Ser Leu Ala Ala Ile Glu Lys Arg Ile Thr
             20                  25                  30

Ile Gln Lys Glu Lys Lys Lys Ser Lys Asp Lys Ala Ala Thr Glu Pro
         35                  40                  45

Gln Pro Arg Pro Gln Leu Asp Leu Lys Ala Ser Arg Lys Leu Pro Lys
     50                  55                  60

Leu Tyr Gly Asp Val Pro Pro Asp Leu Ile Ala Lys Pro Leu Glu Asp
 65                  70                  75                  80

Leu Asp Pro Phe Tyr Lys Asp His Lys Thr Phe Met Val Leu Asn Lys
                 85                  90                  95

Lys Arg Thr Ile Tyr Arg Phe Ser Ala Lys Arg Ala Leu Phe Ile Leu
            100                 105                 110
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Gly Pro Phe Asn Pro Ile Arg Ser Phe Met Ile Arg Ile Ser Val His
        115                 120                 125

Ser Val Phe Ser Met Phe Ile Ile Cys Thr Val Ile Ile Asn Cys Met
    130                 135                 140

Phe Met Ala Asn Asn Ser Ser Val Asp Ser Arg Pro Ser Ser Asn Ile
145                 150                 155                 160

Pro Glu Tyr Val Phe Ile Gly Ile Tyr Val Leu Glu Ala Val Ile Lys
                165                 170                 175

Ile Leu Ala Arg Gly Phe Ile Val Asp Glu Phe Ser Tyr Leu Arg Asp
            180                 185                 190

Pro Trp Asn Trp Leu Asp Phe Ile Val Ile Gly Thr Ala Ile Ala Pro
        195                 200                 205

Cys Phe Leu Gly Asn Lys Val Asn Asn Leu Ser Thr Leu Arg Thr Phe
    210                 215                 220

Arg Val Leu Arg Ala Leu Lys Ala Ile Ser Val Ile Ser Gly Leu Lys
225                 230                 235                 240

Val Ile Val Gly Ala Leu Leu Arg Ser Val Lys Lys Leu Val Asp Val
                245                 250                 255

Met Val Leu Thr Leu Phe Cys Leu Ser Ile Phe Ala Leu Val Gly Gln
            260                 265                 270

Gln Leu Phe Met Gly Ile Leu Ser Gln Lys Cys Ile Lys Asp Asp Cys
        275                 280                 285

Gly Pro Asn Ala Phe Ser Asn Lys Asp Cys Phe Val Lys Glu Asn Asp
    290                 295                 300

Ser Glu Asp Phe Ile Met Cys Gly Asn Trp Leu Gly Arg Arg Ser Cys
305                 310                 315                 320

Pro Asp Gly Ser Thr Cys Asn Lys Thr Thr Phe Asn Pro Asp Tyr Asn
                325                 330                 335

Tyr Thr Asn Phe Asp Ser Phe Gly Trp Ser Phe Leu Ala Met Phe Arg
            340                 345                 350

Val Met Thr Gln Asp Ser Trp Glu Lys Leu Tyr Arg Gln Ile Leu Arg
        355                 360                 365

Thr Ser Gly Ile Tyr Phe Val Phe Phe Phe Val Val Val Ile Phe Leu
    370                 375                 380

Gly Ser Phe Tyr Leu Leu Asn Leu Thr Leu Ala Val Val Thr Met Ala
385                 390                 395                 400

Tyr Glu Glu Gln Asn Arg Asn Val Ala Ala Glu Thr Glu Ala Lys Glu
                405                 410                 415
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Lys Met Phe Gln Glu Ala Gln Gln Leu Leu Arg Glu Glu Lys Glu Ala
            420                 425                 430

Leu Val Ala Met Gly Ile Asp Arg Thr Ser Leu Asn Ser Leu Gln Ala
        435                 440                 445

Ser Ser Phe Ser Pro Lys Lys Arg Lys Phe Phe Gly Ser Lys Thr Arg
    450                 455                 460

Lys Ser Phe Phe Met Arg Gly Ser Lys Thr Ala Arg Ala Ser Ala Ser
465                 470                 475                 480

Asp Ser Glu Asp Asp Ala Ser Lys Asn Pro Gln Leu Leu Glu Gln Thr
                485                 490                 495

Lys Arg Leu Ser Gln Asn Leu Pro Val Glu Leu Phe Asp Glu His Val
            500                 505                 510

Asp Pro Leu His Arg Gln Arg Ala Leu Ser Ala Val Ser Ile Leu Thr
        515                 520                 525

Ile Thr Met Gln Glu Gln Glu Lys Ser Gln Glu Pro Cys Phe Pro Cys
    530                 535                 540

Gly Lys Asn Leu Ala Ser Lys Tyr Leu Val Trp Glu Cys Ser Pro Pro
545                 550                 555                 560

Trp Leu Cys Ile Lys Lys Val Leu Gln Thr Ile Met Thr Asp Pro Phe
                565                 570                 575

Thr Glu Leu Ala Ile Thr Ile Cys Ile Ile Val Asn Thr Val Phe Leu
            580                 585                 590

Ala Met Glu His His Asn Met Asp Asn Ser Leu Lys Asp Ile Leu Lys
        595                 600                 605

Ile Gly Asn Trp Val Phe Thr Gly Ile Phe Ile Ala Glu Met Cys Leu
    610                 615                 620

Lys Ile Ile Ala Leu Asp Pro Tyr His Tyr Phe Arg His Gly Trp Asn
625                 630                 635                 640

Ile Phe Asp Ser Ile Val Ala Leu Val Ser Leu Ala Asp Val Leu Phe
                645                 650                 655

His Lys Leu Ser Lys Asn Leu Ser Phe Leu Ala Ser Leu Arg Val Leu
            660                 665                 670

Arg Val Phe Lys Leu Ala Lys Ser Trp Pro Thr Leu Asn Thr Leu Ile
        675                 680                 685

Lys Ile Ile Gly His Ser Val Gly Ala Leu Gly Asn Leu Thr Val Val
    690                 695                 700

Leu Thr Ile Val Val Phe Ile Phe Ser Val Val Gly Met Arg Leu Phe
705                 710                 715                 720
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Gly Ala Lys Phe Asn Lys Thr Cys Ser Thr Ser Pro Glu Ser Leu Arg
                725                 730                 735

Arg Trp His Met Gly Asp Phe Tyr His Ser Phe Leu Val Val Phe Arg
            740                 745                 750

Ile Leu Cys Gly Glu Trp Ile Glu Asn Met Trp Glu Cys Met Gln Glu
        755                 760                 765

Met Glu Gly Ser Pro Leu Cys Val Ile Val Phe Val Leu Ile Met Val
    770                 775                 780

Val Gly Lys Leu Val Val Leu Asn Leu Phe Ile Ala Leu Leu Leu Asn
785                 790                 795                 800

Ser Phe Ser Asn Glu Glu Lys Asp Gly Asn Pro Glu Gly Glu Thr Arg
                805                 810                 815

Lys Thr Lys Val Gln Leu Ala Leu Asp Arg Phe Ser Arg Ala Phe Tyr
            820                 825                 830

Phe Met Ala Arg Ala Leu Gln Asn Phe Cys Cys Lys Arg Cys Arg Arg
        835                 840                 845

Gln Asn Ser Pro Lys Pro Asn Glu Ala Thr Glu Ser Phe Ala Gly Glu
    850                 855                 860

Ser Arg Asp Thr Ala Thr Leu Asp Thr Arg Ser Trp Lys Glu Tyr Asp
865                 870                 875                 880

Ser Glu Met Thr Leu Tyr Thr Gly Gln Ala Gly Ala Pro Leu Ala Pro
                885                 890                 895

Leu Ala Lys Glu Glu Asp Asp Met Glu Cys Cys Gly Glu Cys Asp Ala
            900                 905                 910

Ser Pro Thr Ser Gln Pro Ser Glu Glu Ala Gln Ala Cys Asp Leu Pro
        915                 920                 925

Leu Lys Thr Lys Arg Leu Pro Ser Pro Asp Asp His Gly Val Glu Met
    930                 935                 940

Glu Val Phe Ser Glu Glu Asp Pro Asn Leu Thr Ile Gln Ser Ala Arg
945                 950                 955                 960

Lys Lys Ser Asp Ala Ala Ser Met Leu Ser Glu Cys Ser Thr Ile Asp
                965                 970                 975

Leu Asn Asp Ile Phe Arg Asn Leu Gln Lys Thr Val Ser Pro Gln Lys
            980                 985                 990

Gln Pro Asp Arg Cys Phe Pro Lys Gly Leu Ser Cys Ile Phe Leu Cys
        995                 1000                1005

Cys Lys Thr Ile Lys Lys Lys Ser Pro Trp Val Leu Trp Trp Asn Leu
   1010                1015                1020
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Arg Lys Thr Cys Tyr Gln Ile Val Lys His Ser Trp Phe Glu Ser Phe
1025                1030                1035                1040

Ile Ile Phe Val Ile Leu Leu Ser Ser Gly Ala Leu Ile Phe Glu Asp
                1045                1050                1055

Val Asn Leu Pro Ser Arg Pro Gln Val Glu Lys Leu Leu Lys Cys Thr
            1060                1065                1070

Asp Asn Ile Phe Thr Phe Ile Phe Leu Leu Glu Met Ile Leu Lys Trp
        1075                1080                1085

Val Ala Phe Gly Phe Arg Lys Tyr Phe Thr Ser Ala Trp Cys Trp Leu
    1090                1095                1100

Asp Phe Leu Ile Val Val Val Ser Val Leu Ser Leu Thr Asn Leu Pro
1105                1110                1115                1120

Asn Leu Lys Ser Phe Arg Asn Leu Arg Ala Leu Arg Pro Leu Arg Ala
                1125                1130                1135

Leu Ser Gln Phe Glu Gly Met Lys Val Val Val Asn Ala Leu Met Ser
            1140                1145                1150

Ala Ile Pro Ala Ile Leu Asn Val Leu Leu Val Cys Leu Ile Phe Trp
        1155                1160                1165

Leu Ile Phe Cys Ile Leu Gly Val Asn Phe Phe Ser Gly Lys Phe Gly
    1170                1175                1180

Arg Cys Ile Asn Gly Thr Asp Ile Asn Lys Tyr Phe Asn Ala Ser Asn
1185                1190                1195                1200

Val Pro Asn Gln Ser Gln Cys Leu Val Ser Asn Tyr Thr Trp Lys Val
                1205                1210                1215

Pro Asn Val Asn Phe Asp Asn Val Gly Asn Ala Tyr Leu Ala Leu Leu
            1220                1225                1230

Gln Val Ala Thr Tyr Lys Gly Trp Leu Asp Ile Met Asn Ala Ala Val
        1235                1240                1245

Asp Ser Arg Gly Lys Asp Glu Gln Pro Ala Phe Glu Ala Asn Leu Tyr
    1250                1255                1260

Ala Tyr Leu Tyr Phe Val Val Phe Ile Ile Phe Gly Ser Phe Phe Thr
1265                1270                1275                1280

Leu Asn Leu Phe Ile Gly Val Ile Ile Asp Asn Phe Asn Gln Gln Gln
                1285                1290                1295

Lys Lys Leu Gly Gly Gln Asp Ile Phe Met Thr Glu Glu Gln Lys Lys
            1300                1305                1310

Tyr Tyr Asn Ala Met Lys Lys Leu Gly Thr Lys Lys Pro Gln Lys Pro
        1315                1320                1325
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Ile Pro Arg Pro Leu Asn Lys Cys Gln Ala Phe Val Phe Asp Leu Val
1330 1335 1340

Thr Ser Gln Val Phe Asp Val Ile Ile Leu Gly Leu Ile Val Thr Asn
1345 1350 1355 1360

Met Ile Ile Met Met Ala Glu Ser Glu Gly Gln Pro Asn Glu Val Lys
1365 1370 1375

Lys Ile Phe Asp Ile Leu Asn Ile Val Phe Val Val Ile Phe Thr Val
1380 1385 1390

Glu Cys Leu Ile Lys Val Phe Ala Leu Arg Gln His Tyr Phe Thr Asn
1395 1400 1405

Gly Trp Asn Leu Phe Asp Cys Val Val Val Val Leu Ser Ile Ile Ser
1410 1415 1420

Thr Leu Val Ser Gly Leu Glu Asn Ser Asn Val Phe Pro Pro Thr Leu
1425 1430 1435 1440

Phe Arg Ile Val Arg Leu Ala Arg Ile Gly Arg Ile Leu Arg Leu Val
1445 1450 1455

Arg Ala Ala Arg Gly Ile Arg Thr Leu Leu Phe Ala Leu Met Met Ser
1460 1465 1470

Leu Pro Ser Leu Phe Asn Ile Gly Leu Leu Leu Phe Leu Val Met Phe
1475 1480 1485

Ile Tyr Ala Ile Phe Gly Met Asn Trp Phe Ser Lys Val Lys Arg Gly
1490 1495 1500

Ser Gly Ile Asp Asp Ile Phe Asn Phe Asp Thr Phe Ser Gly Ser Met
1505 1510 1515 1520

Leu Cys Leu Phe Gln Ile Thr Thr Ser Ala Gly Trp Asp Ala Leu Leu
1525 1530 1535

Asn Pro Met Leu Glu Ser Lys Ala Ser Cys Asn Ser Ser Ser Gln Glu
1540 1545 1550

Ser Cys Gln Gln Pro Gln Ile Ala Ile Val Tyr Phe Val Ser Tyr Ile
1555 1560 1565

Ile Ile Ser Phe Leu Ile Val Val Asn Met Tyr Ile Ala Val Ile Leu
1570 1575 1580

Glu Asn Phe Asn Thr Ala Thr Glu Glu Ser Glu Asp Pro Leu Gly Glu
1585 1590 1595 1600

Asp Asp Phe Glu Ile Phe Tyr Glu Ile Trp Glu Lys Phe Asp Pro Glu
1605 1610 1615

Ala Thr Gln Phe Ile Gln Tyr Ser Ser Leu Ser Asp Phe Ala Asp Ala
1620 1625 1630

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Leu Pro Glu Pro Leu Arg Val Ala Lys Pro Asn Arg Phe Gln Phe Leu
       1635                1640                1645

Met Met Asp Leu Pro Met Val Met Gly Asp Arg Leu His Cys Met Asp
   1650                1655                1660

Val Leu Phe Ala Phe Thr Thr Arg Val Leu Gly Asn Ser Ser Gly Leu
1665                1670                1675                1680

Asp Thr Met Lys Ala Met Met Glu Glu Lys Phe Met Glu Ala Asn Pro
               1685                1690                1695

Phe Lys Lys Leu Tyr Glu Pro Ile Val Thr Thr Thr Lys Arg Lys Glu
           1700                1705                1710

Glu Glu Glu Cys Ala Ala Val Ile Gln Arg Ala Tyr Arg Arg His Met
       1715                1720                1725

Glu Lys Met Ile Lys Leu Lys Leu Lys Gly Arg Ser Ser Ser Ser Leu
   1730                1735                1740

Gln Val Phe Cys Asn Gly Asp Leu Ser Ser Leu Asp Val Pro Lys Ile
1745                1750                1755                1760

Lys Val His Cys Asp
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Ser Ile Val Ile Gly Ile Ala Ile Val Ser Tyr Ile Pro Gly Ile Thr
  1               5                  10                  15

atc aaa cta ttg ccc ctg cgt acc ttc cgt gtg ttc aga gct ttg aaa   96
Ile Lys Leu Leu Pro Leu Arg Thr Phe Arg Val Phe Arg Ala Leu Lys
             20                  25                  30

gca att tca gta gtt tca cgt ctg aag gtc atc gtg ggg gcc ttg cta   144
Ala Ile Ser Val Val Ser Arg Leu Lys Val Ile Val Gly Ala Leu Leu
         35                  40                  45
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cgc tct gtg aag aag ctg gtc aac gtg att atc ctc acc ttc ttt tgc   192
Arg Ser Val Lys Lys Leu Val Asn Val Ile Ile Leu Thr Phe Phe Cys
     50                  55                  60

ctc agc atc ttt gcc ctg gta ggt cag cag ctc ttc atg gga agt ctg   240
Leu Ser Ile Phe Ala Leu Val Gly Gln Gln Leu Phe Met Gly Ser Leu
 65                  70                  75                  80

aac ctg aaa tgc atc tcg agg gac tgt aaa aat atc agt aac ccg gaa   288
Asn Leu Lys Cys Ile Ser Arg Asp Cys Lys Asn Ile Ser Asn Pro Glu
                85                  90                  95

gct tat gac cat tgc ttt gaa aag aaa gaa aat tca cct gaa ttc aaa   336
Ala Tyr Asp His Cys Phe Glu Lys Lys Glu Asn Ser Pro Glu Phe Lys
            100                 105                 110

atg tgt ggc atc tgg atg ggt aac agt gcc tgt tcc ata caa tat gaa   384
Met Cys Gly Ile Trp Met Gly Asn Ser Ala Cys Ser Ile Gln Tyr Glu
        115                 120                 125

tgt aag cac acc aaa att aat cct gac tat aat tat acg aat ttt gac   432
Cys Lys His Thr Lys Ile Asn Pro Asp Tyr Asn Tyr Thr Asn Phe Asp
    130                 135                 140

aac ttt ggc tgg tct ttt ctt gcc atg ttc cgg ctg atg acc caa gat   480
Asn Phe Gly Trp Ser Phe Leu Ala Met Phe Arg Leu Met Thr Gln Asp
145                 150                 155                 160

tcc tgg gag aag ctt tat caa cag acc ctg cgt act act ggg ctc tac   528
Ser Trp Glu Lys Leu Tyr Gln Gln Thr Leu Arg Thr Thr Gly Leu Tyr
                165                 170                 175

tca gtc ttc ttc ttc att gtg gtc att ttc ctg ggc tcc ttc tac ctg   576
Ser Val Phe Phe Phe Ile Val Val Ile Phe Leu Gly Ser Phe Tyr Leu
            180                 185                 190

att aac tta acc ctg gct gtt gtt acc atg gca tat gag gag cag aac   624
Ile Asn Leu Thr Leu Ala Val Val Thr Met Ala Tyr Glu Glu Gln Asn
        195                 200                 205

aag aat gta gct gca gag ata gag gcc aag gaa aag atg ttt cag gaa   672
Lys Asn Val Ala Ala Glu Ile Glu Ala Lys Glu Lys Met Phe Gln Glu
    210                 215                 220

gcc cag cag ctg tta aag gag gaa aag gag gct ctg gtt gcc atg gga   720
Ala Gln Gln Leu Leu Lys Glu Glu Lys Glu Ala Leu Val Ala Met Gly
225                 230                 235                 240

att gac aga agt tca ctt act tcc ctt gaa aca tca tat ttt acc cca   768
Ile Asp Arg Ser Ser Leu Thr Ser Leu Glu Thr Ser Tyr Phe Thr Pro
                245                 250                 255

aaa aag aga aag ctc ttt ggt aat aag aaa agg aag tcc ttc ttt ttg   816
Lys Lys Arg Lys Leu Phe Gly Asn Lys Lys Arg Lys Ser Phe Phe Leu
            260                 265                 270
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aga gag tct ggg aaa gac cag cct cct ggg tca gat tct gat gaa gat   864
Arg Glu Ser Gly Lys Asp Gln Pro Pro Gly Ser Asp Ser Asp Glu Asp
        275                 280                 285

tgc caa aaa aag cca cag ctc cta gag caa acc aaa cga ctg tcc cag   912
Cys Gln Lys Lys Pro Gln Leu Leu Glu Gln Thr Lys Arg Leu Ser Gln
    290                 295                 300

aat cta tca ytg gac cac ttt gat gag cat gga gat cct ctc caa agg   960
Asn Leu Ser Xaa Asp His Phe Asp Glu His Gly Asp Pro Leu Gln Arg
305                 310                 315                 320

cag aga gca ctg agt gct gtc agc atc ctc acc atc acc atg aag gaa   1008
Gln Arg Ala Leu Ser Ala Val Ser Ile Leu Thr Ile Thr Met Lys Glu
                325                 330                 335

caa gaa aaa tca caa gag cct tgt ctc cct tgt gga gaa aac ctg gca   1056
Gln Glu Lys Ser Gln Glu Pro Cys Leu Pro Cys Gly Glu Asn Leu Ala
            340                 345                 350

tcc aag tac ctc gtg tgg aac tgt tgc ccc cag tgg ctg tgc gtt aag   1104
Ser Lys Tyr Leu Val Trp Asn Cys Cys Pro Gln Trp Leu Cys Val Lys
        355                 360                 365

aag gtc ctg aga act gtg atg act gac ccg ttt act gag ctg gcc atc   1152
Lys Val Leu Arg Thr Val Met Thr Asp Pro Phe Thr Glu Leu Ala Ile
    370                 375                 380

acc atc tgc atc atc atc aac act gtc ttc ttg gcc atg gag cat cac   1200
Thr Ile Cys Ile Ile Ile Asn Thr Val Phe Leu Ala Met Glu His His
385                 390                 395                 400

aag atg gag gcc agt ttt gag aag atg ttg aat ata ggg aat ttg gtt   1248
Lys Met Glu Ala Ser Phe Glu Lys Met Leu Asn Ile Gly Asn Leu Val
                405                 410                 415

ttc act agc att ttt ata gca gaa atg tgc cta aaa atc att gcg ctc   1296
Phe Thr Ser Ile Phe Ile Ala Glu Met Cys Leu Lys Ile Ile Ala Leu
            420                 425                 430

gat ccc tac cac tac ttt cgc cga ggc tgg aac att ttt gac agc att   1344
Asp Pro Tyr His Tyr Phe Arg Arg Gly Trp Asn Ile Phe Asp Ser Ile
        435                 440                 445

gtt gct ctt ctg agt ttt gca gat gta atg aac tgt gta ctt caa aag   1392
Val Ala Leu Leu Ser Phe Ala Asp Val Met Asn Cys Val Leu Gln Lys
    450                 455                 460

aga agc tgg cca ttc ttg cgt tcc ttc aga gtg ctc agg gtc ttc aag   1440
Arg Ser Trp Pro Phe Leu Arg Ser Phe Arg Val Leu Arg Val Phe Lys
465                 470                 475                 480

tta gcc aaa tcc tgg cca act ttg aac aca cta att aag ata atc ggc   1488
Leu Ala Lys Ser Trp Pro Thr Leu Asn Thr Leu Ile Lys Ile Ile Gly
                485                 490                 495
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aac tct gtc gga gcc ctt gga agc ctg act gtg gtc ctg gtc att gtg   1536
Asn Ser Val Gly Ala Leu Gly Ser Leu Thr Val Val Leu Val Ile Val
            500                 505                 510

atc ttt att ttc tca gta gtt ggc atg cag ctt ttt ggc cgt agc ttc   1584
Ile Phe Ile Phe Ser Val Val Gly Met Gln Leu Phe Gly Arg Ser Phe
        515                 520                 525

aat tcc caa aag agt cca aaa ctc tgt aac ccg aca ggc ccg aca gtc   1632
Asn Ser Gln Lys Ser Pro Lys Leu Cys Asn Pro Thr Gly Pro Thr Val
    530                 535                 540

tca tgt tta cgg cac tgg cac atg ggg gat ttc tgg cac tcc ttc cta   1680
Ser Cys Leu Arg His Trp His Met Gly Asp Phe Trp His Ser Phe Leu
545                 550                 555                 560

gtg gta ttc cgc atc ctc tgc ggg gaa tgg atc gaa aat atg tgg gaa   1728
Val Val Phe Arg Ile Leu Cys Gly Glu Trp Ile Glu Asn Met Trp Glu
                565                 570                 575

tgt atg caa gaa gcg aat gca tca tca tca ttg tgt gtt att gtc ttc   1776
Cys Met Gln Glu Ala Asn Ala Ser Ser Ser Leu Cys Val Ile Val Phe
            580                 585                 590

ata ttg atc acg gtg ata gga aaa ctt gtg gtg ctc aac ctc ttc att   1824
Ile Leu Ile Thr Val Ile Gly Lys Leu Val Val Leu Asn Leu Phe Ile
        595                 600                 605

gcc tta ctg ctc aat tcc ttt agc aat gag gaa aga aat gga aac tta   1872
Ala Leu Leu Leu Asn Ser Phe Ser Asn Glu Glu Arg Asn Gly Asn Leu
    610                 615                 620

gaa gga gag gcc agg aaa act aaa gtc cag tta gca ctg gat cga ttc   1920
Glu Gly Glu Ala Arg Lys Thr Lys Val Gln Leu Ala Leu Asp Arg Phe
625                 630                 635                 640

cgc cgg gct ttt tgt ttt gtg aga cac act ctt gag cat ttc tgt cac   1968
Arg Arg Ala Phe Cys Phe Val Arg His Thr Leu Glu His Phe Cys His
                645                 650                 655

aag tgg tgc agg aag caa aac tta cca cag caa aaa gag gtg gca gga   2016
Lys Trp Cys Arg Lys Gln Asn Leu Pro Gln Gln Lys Glu Val Ala Gly
            660                 665                 670

ggc tgt gct gca caa agc aaa gac atc att ccc ctg gtc atg gag atg   2064
Gly Cys Ala Ala Gln Ser Lys Asp Ile Ile Pro Leu Val Met Glu Met
        675                 680                 685

aaa agg ggc tca gag acc cag gag gag ctt ggt ata cta acc tct gta   2112
Lys Arg Gly Ser Glu Thr Gln Glu Glu Leu Gly Ile Leu Thr Ser Val
    690                 695                 700

cca aag acc ctg ggc gtc agg cat gat tgg act tgg ttg gca cca ctt   2160
Pro Lys Thr Leu Gly Val Arg His Asp Trp Thr Trp Leu Ala Pro Leu
705                 710                 715                 720
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gcg gag gag gaa gat gac gtt gaa ttt tct ggt gaa gat aat gca cag   2208
Ala Glu Glu Glu Asp Asp Val Glu Phe Ser Gly Glu Asp Asn Ala Gln
                725                 730                 735

cgc atc aca caa cct gag cct gaa caa cag gcc tat gag ctc cat cag   2256
Arg Ile Thr Gln Pro Glu Pro Glu Gln Gln Ala Tyr Glu Leu His Gln
            740                 745                 750

gag aac aag aag ccc acg agc cag aga gtt caa agt gtg gaa att gac   2304
Glu Asn Lys Lys Pro Thr Ser Gln Arg Val Gln Ser Val Glu Ile Asp
        755                 760                 765

atg ttc tct gaa gat gag cct cat ctg acc ata cag gat ccc cga aag   2352
Met Phe Ser Glu Asp Glu Pro His Leu Thr Ile Gln Asp Pro Arg Lys
    770                 775                 780

aag tct gat gtt acc agt ata cta tca gaa tgt agc acc att gat ctt   2400
Lys Ser Asp Val Thr Ser Ile Leu Ser Glu Cys Ser Thr Ile Asp Leu
785                 790                 795                 800

cag gat ggc ttt gga tgg tta cct gag atg gtt ccc aaa aag caa cca   2448
Gln Asp Gly Phe Gly Trp Leu Pro Glu Met Val Pro Lys Lys Gln Pro
                805                 810                 815

gag aga tgt ttg ccc aaa ggc ttt ggt tgc tgc ttt cca tgc tgt agc   2496
Glu Arg Cys Leu Pro Lys Gly Phe Gly Cys Cys Phe Pro Cys Cys Ser
            820                 825                 830

gtg gac aag aga aag cct ccc tgg gtc att tgg tgg aac ctg cgg aaa   2544
Val Asp Lys Arg Lys Pro Pro Trp Val Ile Trp Trp Asn Leu Arg Lys
        835                 840                 845

acc tgc tac caa ata gtg aaa cac agc tgg ttt gag agc ttt att atc   2592
Thr Cys Tyr Gln Ile Val Lys His Ser Trp Phe Glu Ser Phe Ile Ile
    850                 855                 860

ttt gtg att ctg ctg agc agt ggg gca ctg ata ttt gaa gat gtt cac   2640
Phe Val Ile Leu Leu Ser Ser Gly Ala Leu Ile Phe Glu Asp Val His
865                 870                 875                 880

ctt gag aac caa ccc aaa atc caa gaa tta cta aat tgt act gac att   2688
Leu Glu Asn Gln Pro Lys Ile Gln Glu Leu Leu Asn Cys Thr Asp Ile
                885                 890                 895

att ttt aca cat att ttt atc ctg gag atg gta cta aaa tgg gta gcc   2736
Ile Phe Thr His Ile Phe Ile Leu Glu Met Val Leu Lys Trp Val Ala
            900                 905                 910

ttc gga ttt gga aag tat ttc acc agt gcc tgg tgc tgc ctt gat ttc   2784
Phe Gly Phe Gly Lys Tyr Phe Thr Ser Ala Trp Cys Cys Leu Asp Phe
        915                 920                 925

atc att gtg att gtc tct gtg acc acc ctc att aac tta atg gaa ttg   2832
Ile Ile Val Ile Val Ser Val Thr Thr Leu Ile Asn Leu Met Glu Leu
    930                 935                 940
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aag tcc ttc cgg act cta cga gca ctg agg cct ctt cgt gcg ctg tcc   2880
Lys Ser Phe Arg Thr Leu Arg Ala Leu Arg Pro Leu Arg Ala Leu Ser
945                 950                 955                 960

cag ttt gaa gga atg aag gtg gtg gtc aat gct ctc ata ggt gcc ata   2928
Gln Phe Glu Gly Met Lys Val Val Val Asn Ala Leu Ile Gly Ala Ile
                965                 970                 975

cct gcc att ctg aat gtt ttg ctt gtc tgc ctc att ttc tgg ctc gta   2976
Pro Ala Ile Leu Asn Val Leu Leu Val Cys Leu Ile Phe Trp Leu Val
            980                 985                 990

ttt tgt att ctg gga gta tac ttc ttt tct gga aaa ttt ggg aaa tgc   3024
Phe Cys Ile Leu Gly Val Tyr Phe Phe Ser Gly Lys Phe Gly Lys Cys
        995                1000                1005

att aat gga aca gac tca gtt ata aat tat acc atc att aca aat aaa   3072
Ile Asn Gly Thr Asp Ser Val Ile Asn Tyr Thr Ile Ile Thr Asn Lys
   1010                1015                1020

agt caa tgt gaa agt ggc aat ttc tct tgg atc aac cag aaa gtc aac   3120
Ser Gln Cys Glu Ser Gly Asn Phe Ser Trp Ile Asn Gln Lys Val Asn
1025                1030                1035                1040

ttt gac aat gtg gga aat gct tac ctc gct ctg ctg caa gtg gca aca   3168
Phe Asp Asn Val Gly Asn Ala Tyr Leu Ala Leu Leu Gln Val Ala Thr
                1045                1050                1055

ttt aag ggc tgg atg gat att ata tat gca gct gtt gat tcc aca gag   3216
Phe Lys Gly Trp Met Asp Ile Ile Tyr Ala Ala Val Asp Ser Thr Glu
            1060                1065                1070

aaa gaa caa cag cca gag ttt gag agc aat tca ctc ggt tac att tac   3264
Lys Glu Gln Gln Pro Glu Phe Glu Ser Asn Ser Leu Gly Tyr Ile Tyr
        1075                1080                1085

ttc gta gtc ttt atc atc ttt ggc tca ttc ttc act ctg aat ctc ttc   3312
Phe Val Val Phe Ile Ile Phe Gly Ser Phe Phe Thr Leu Asn Leu Phe
   1090                1095                1100

att ggc gtt atc att gac aac ttc aac caa cag cag aaa aag tta ggt   3360
Ile Gly Val Ile Ile Asp Asn Phe Asn Gln Gln Gln Lys Lys Leu Gly
1105                1110                1115                1120

ggc caa gac att ttt atg aca gaa gaa cag aag aaa tac tat aat gca   3408
Gly Gln Asp Ile Phe Met Thr Glu Glu Gln Lys Lys Tyr Tyr Asn Ala
                1125                1130                1135

atg aaa aaa tta gga tcc aaa aaa cct caa aaa ccc att cca cgg cct   3456
Met Lys Lys Leu Gly Ser Lys Lys Pro Gln Lys Pro Ile Pro Arg Pro
            1140                1145                1150

ctg aac aaa tgt caa ggt ctc gtg ttc gac ata gtc aca agc cag atc   3504
Leu Asn Lys Cys Gln Gly Leu Val Phe Asp Ile Val Thr Ser Gln Ile
        1155                1160                1165
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ttt gac atc atc atc ata agt ctc att atc cta aac atg att agc atg   3552
Phe Asp Ile Ile Il  Ile Ser Leu Ile Ile Leu Asn Met Ile Ser Met
   1170                1175                1180

atg gct gaa tca tac aac caa ccc aaa gcc atg aaa tcc atc ctt gac   3600
Met Ala Glu Ser Tyr Asn Gln Pro Lys Ala Met Lys Ser Ile Leu Asp
1185                1190                1195                1200

cat ctc aac tgg gtc ttt gtg gtc atc ttt acg tta gaa tgt ctc atc   3648
His Leu Asn Trp Val Phe Val Val Ile Phe Thr Leu Glu Cys Leu Ile
               1205                1210                1215

aaa atc ttt gct ttg agg caa tac tac ttc acc aat ggc tgg aat tta   3696
Lys Ile Phe Ala Leu Arg Gln Tyr Tyr Phe Thr Asn Gly Trp Asn Leu
          1220                1225                1230

ttt ga                                                           3701
Phe
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  1               5                  10                  15

Ile Lys Leu Leu Pro Leu Arg Thr Phe Arg Val Phe Arg Ala Leu Lys
             20                  25                  30

Ala Ile Ser Val Val Ser Arg Leu Lys Val Ile Val Gly Ala Leu Leu
         35                  40                  45

Arg Ser Val Lys Lys Leu Val Asn Val Ile Ile Leu Thr Phe Phe Cys
     50                  55                  60

Leu Ser Ile Phe Ala Leu Val Gly Gln Gln Leu Phe Met Gly Ser Leu
 65                  70                  75                  80

Asn Leu Lys Cys Ile Ser Arg Asp Cys Lys Asn Ile Ser Asn Pro Glu
                 85                  90                  95

Ala Tyr Asp His Cys Phe Glu Lys Lys Glu Asn Ser Pro Glu Phe Lys
            100                 105                 110

Met Cys Gly Ile Trp Met Gly Asn Ser Ala Cys Ser Ile Gln Tyr Glu
        115                 120                 125

Cys Lys His Thr Lys Ile Asn Pro Asp Tyr Asn Tyr Thr Asn Phe Asp
    130                 135                 140

Asn Phe Gly Trp Ser Phe Leu Ala Met Phe Arg Leu Met Thr Gln Asp
145                 150                 155                 160
```

```
Ser Trp Glu Lys Leu Tyr Gln Gln Thr Leu Arg Thr Thr Gly Leu Tyr
                165                 170                 175

Ser Val Phe Phe Phe Ile Val Val Ile Phe Leu Gly Ser Phe Tyr Leu
            180                 185                 190

Ile Asn Leu Thr Leu Ala Val Val Thr Met Ala Tyr Glu Glu Gln Asn
        195                 200                 205

Lys Asn Val Ala Ala Glu Ile Glu Ala Lys Glu Lys Met Phe Gln Glu
    210                 215                 220

Ala Gln Gln Leu Leu Lys Glu Glu Lys Glu Ala Leu Val Ala Met Gly
225                 230                 235                 240

Ile Asp Arg Ser Ser Leu Thr Ser Leu Glu Thr Ser Tyr Phe Thr Pro
                245                 250                 255

Lys Lys Arg Lys Leu Phe Gly Asn Lys Lys Arg Lys Ser Phe Phe Leu
            260                 265                 270

Arg Glu Ser Gly Lys Asp Gln Pro Pro Gly Ser Asp Ser Asp Glu Asp
        275                 280                 285

Cys Gln Lys Lys Pro Gln Leu Leu Glu Gln Thr Lys Arg Leu Ser Gln
    290                 295                 300

Asn Leu Ser Xaa Asp His Phe Asp Glu His Gly Asp Pro Leu Gln Arg
305                 310                 315                 320

Gln Arg Ala Leu Ser Ala Val Ser Ile Leu Thr Ile Thr Met Lys Glu
                325                 330                 335

Gln Glu Lys Ser Gln Glu Pro Cys Leu Pro Cys Gly Glu Asn Leu Ala
            340                 345                 350

Ser Lys Tyr Leu Val Trp Asn Cys Cys Pro Gln Trp Leu Cys Val Lys
        355                 360                 365

Lys Val Leu Arg Thr Val Met Thr Asp Pro Phe Thr Glu Leu Ala Ile
    370                 375                 380

Thr Ile Cys Ile Ile Ile Asn Thr Val Phe Leu Ala Met Glu His His
385                 390                 395                 400

Lys Met Glu Ala Ser Phe Glu Lys Met Leu Asn Ile Gly Asn Leu Val
                405                 410                 415

Phe Thr Ser Ile Phe Ile Ala Glu Met Cys Leu Lys Ile Ile Ala Leu
            420                 425                 430

Asp Pro Tyr His Tyr Phe Arg Arg Gly Trp Asn Ile Phe Asp Ser Ile
        435                 440                 445

Val Ala Leu Leu Ser Phe Ala Asp Val Met Asn Cys Val Leu Gln Lys
    450                 455                 460
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Arg Ser Trp Pro Phe Leu Arg Ser Phe Arg Val Leu Arg Val Phe Lys
465                 470                 475                 480

Leu Ala Lys Ser Trp Pro Thr Leu Asn Thr Leu Ile Lys Ile Ile Gly
                485                 490                 495

Asn Ser Val Gly Ala Leu Gly Ser Leu Thr Val Val Leu Val Ile Val
            500                 505                 510

Ile Phe Ile Phe Ser Val Val Gly Met Gln Leu Phe Gly Arg Ser Phe
        515                 520                 525

Asn Ser Gln Lys Ser Pro Lys Leu Cys Asn Pro Thr Gly Pro Thr Val
    530                 535                 540

Ser Cys Leu Arg His Trp His Met Gly Asp Phe Trp His Ser Phe Leu
545                 550                 555                 560

Val Val Phe Arg Ile Leu Cys Gly Glu Trp Ile Glu Asn Met Trp Glu
                565                 570                 575

Cys Met Gln Glu Ala Asn Ala Ser Ser Ser Leu Cys Val Ile Val Phe
            580                 585                 590

Ile Leu Ile Thr Val Ile Gly Lys Leu Val Val Leu Asn Leu Phe Ile
        595                 600                 605

Ala Leu Leu Leu Asn Ser Phe Ser Asn Glu Glu Arg Asn Gly Asn Leu
    610                 615                 620

Glu Gly Glu Ala Arg Lys Thr Lys Val Gln Leu Ala Leu Asp Arg Phe
625                 630                 635                 640

Arg Arg Ala Phe Cys Phe Val Arg His Thr Leu Glu His Phe Cys His
                645                 650                 655

Lys Trp Cys Arg Lys Gln Asn Leu Pro Gln Gln Lys Glu Val Ala Gly
            660                 665                 670

Gly Cys Ala Ala Gln Ser Lys Asp Ile Ile Pro Leu Val Met Glu Met
        675                 680                 685

Lys Arg Gly Ser Glu Thr Gln Glu Glu Leu Gly Ile Leu Thr Ser Val
    690                 695                 700

Pro Lys Thr Leu Gly Val Arg His Asp Trp Thr Trp Leu Ala Pro Leu
705                 710                 715                 720

Ala Glu Glu Glu Asp Asp Val Glu Phe Ser Gly Glu Asp Asn Ala Gln
                725                 730                 735

Arg Ile Thr Gln Pro Glu Pro Glu Gln Gln Ala Tyr Glu Leu His Gln
            740                 745                 750

Glu Asn Lys Lys Pro Thr Ser Gln Arg Val Gln Ser Val Glu Ile Asp
        755                 760                 765
```

```
Met Phe Ser Glu Asp Glu Pro His Leu Thr Ile Gln Asp Pro Arg Lys
    770                 775                 780

Lys Ser Asp Val Thr Ser Ile Leu Ser Glu Cys Ser Thr Ile Asp Leu
785                 790                 795                 800

Gln Asp Gly Phe Gly Trp Leu Pro Glu Met Val Pro Lys Lys Gln Pro
                805                 810                 815

Glu Arg Cys Leu Pro Lys Gly Phe Gly Cys Cys Phe Pro Cys Cys Ser
            820                 825                 830

Val Asp Lys Arg Lys Pro Pro Trp Val Ile Trp Trp Asn Leu Arg Lys
        835                 840                 845

Thr Cys Tyr Gln Ile Val Lys His Ser Trp Phe Glu Ser Phe Ile Ile
    850                 855                 860

Phe Val Ile Leu Leu Ser Ser Gly Ala Leu Ile Phe Glu Asp Val His
865                 870                 875                 880

Leu Glu Asn Gln Pro Lys Ile Gln Glu Leu Leu Asn Cys Thr Asp Ile
                885                 890                 895

Ile Phe Thr His Ile Phe Ile Leu Glu Met Val Leu Lys Trp Val Ala
            900                 905                 910

Phe Gly Phe Gly Lys Tyr Phe Thr Ser Ala Trp Cys Cys Leu Asp Phe
        915                 920                 925

Ile Ile Val Ile Val Ser Val Thr Thr Leu Ile Asn Leu Met Glu Leu
    930                 935                 940

Lys Ser Phe Arg Thr Leu Arg Ala Leu Arg Pro Leu Arg Ala Leu Ser
945                 950                 955                 960

Gln Phe Glu Gly Met Lys Val Val Val Asn Ala Leu Ile Gly Ala Ile
                965                 970                 975

Pro Ala Ile Leu Asn Val Leu Leu Val Cys Leu Ile Phe Trp Leu Val
            980                 985                 990

Phe Cys Ile Leu Gly Val Tyr Phe Phe Ser Gly Lys Phe Gly Lys Cys
        995                1000                1005

Ile Asn Gly Thr Asp Ser Val Ile Asn Tyr Thr Ile Ile Thr Asn Lys
   1010                1015                1020

Ser Gln Cys Glu Ser Gly Asn Phe Ser Trp Ile Asn Gln Lys Val Asn
1025                1030                1035                1040

Phe Asp Asn Val Gly Asn Ala Tyr Leu Ala Leu Leu Gln Val Ala Thr
                1045                1050                1055

Phe Lys Gly Trp Met Asp Ile Ile Tyr Ala Ala Val Asp Ser Thr Glu
            1060                1065                1070
```

```
Lys Glu Gln Gln Pro Glu Phe Glu Ser Asn Ser Leu Gly Tyr Ile Tyr
       1075                1080                1085

Phe Val Val Phe Ile Ile Phe Gly Ser Phe Phe Thr Leu Asn Leu Phe
   1090                1095                1100

Ile Gly Val Ile Ile Asp Asn Phe Asn Gln Gln Gln Lys Lys Leu Gly
1105                1110                1115                1120

Gly Gln Asp Ile Phe Met Thr Glu Glu Gln Lys Lys Tyr Tyr Asn Ala
               1125                1130                1135

Met Lys Lys Leu Gly Ser Lys Lys Pro Gln Lys Pro Ile Pro Arg Pro
           1140                1145                1150

Leu Asn Lys Cys Gln Gly Leu Val Phe Asp Ile Val Thr Ser Gln Ile
       1155                1160                1165

Phe Asp Ile Ile Ile Ile Ser Leu Ile Ile Leu Asn Met Ile Ser Met
   1170                1175                1180

Met Ala Glu Ser Tyr Asn Gln Pro Lys Ala Met Lys Ser Ile Leu Asp
1185                1190                1195                1200

His Leu Asn Trp Val Phe Val Val Ile Phe Thr Leu Glu Cys Leu Ile
               1205                1210                1215

Lys Ile Phe Ala Leu Arg Gln Tyr Tyr Phe Thr Asn Gly Trp Asn Leu
           1220                1225                1230

Phe
```

<210> 8
<211> 1243
<212> PRT
<213> Homo sapiens

<220>
<223> partial human NaN amino acid seq.

<400> 8

```
Ser Ile Val Ile Gly Ile Ala Ile Val Ser Tyr Ile Pro Gly Ile Thr
  1               5                  10                  15

Ile Lys Leu Leu Pro Leu Arg Thr Phe Arg Val Phe Arg Ala Leu Lys
             20                  25                  30

Ala Ile Ser Val Val Ser Arg Leu Lys Val Ile Val Gly Ala Leu Leu
         35                  40                  45

Arg Ser Val Lys Lys Leu Val Asn Val Ile Ile Leu Thr Phe Phe Cys
     50                  55                  60
```

```
Leu Ser Ile Phe Ala Leu Val Gly Gln Gln Leu Phe Met Gly Ser Leu
 65                  70                  75                  80

Asn Leu Lys Cys Ile Ser Arg Asp Cys Lys Asn Ile Ser Asn Pro Glu
                 85                  90                  95

Ala Tyr Asp His Cys Phe Glu Lys Lys Glu Asn Ser Pro Glu Phe Lys
            100                 105                 110

Met Cys Gly Ile Trp Met Gly Asn Ser Ala Cys Ser Ile Gln Tyr Glu
        115                 120                 125

Cys Lys His Thr Lys Ile Asn Pro Asp Tyr Asn Tyr Thr Asn Phe Asp
    130                 135                 140

Asn Phe Gly Trp Ser Phe Leu Ala Met Phe Arg Leu Met Thr Gln Asp
145                 150                 155                 160

Ser Trp Glu Lys Leu Tyr Gln Gln Thr Leu Arg Thr Thr Gly Leu Tyr
                165                 170                 175

Ser Val Phe Phe Phe Ile Val Val Ile Phe Leu Gly Ser Phe Tyr Leu
            180                 185                 190

Ile Asn Leu Thr Leu Ala Val Val Thr Met Ala Tyr Glu Glu Gln Asn
        195                 200                 205

Lys Asn Val Ala Ala Glu Ile Glu Ala Lys Glu Lys Met Phe Gln Glu
    210                 215                 220

Ala Gln Gln Leu Leu Lys Glu Glu Lys Glu Ala Leu Val Ala Met Gly
225                 230                 235                 240

Ile Asp Arg Ser Ser Leu Thr Ser Leu Glu Thr Ser Tyr Phe Thr Pro
                245                 250                 255

Lys Lys Arg Lys Leu Phe Gly Asn Lys Lys Arg Lys Ser Phe Phe Leu
            260                 265                 270

Arg Glu Ser Gly Lys Asp Gln Pro Pro Gly Ser Asp Ser Asp Glu Asp
        275                 280                 285

Cys Gln Lys Lys Pro Gln Leu Leu Glu Gln Thr Lys Arg Leu Ser Gln
    290                 295                 300

Asn Leu Ser Leu Asp His Phe Asp Glu His Gly Asp Pro Leu Gln Arg
305                 310                 315                 320

Gln Arg Ala Leu Ser Ala Val Ser Ile Leu Thr Ile Thr Met Lys Glu
                325                 330                 335

Gln Glu Lys Ser Gln Glu Pro Cys Leu Pro Cys Gly Glu Asn Leu Ala
            340                 345                 350

Ser Lys Tyr Leu Val Trp Asn Cys Cys Pro Gln Trp Leu Cys Val Lys
        355                 360                 365
```

```
Lys Val Leu Arg Thr Val Met Thr Asp Pro Phe Thr Glu Leu Ala Ile
    370                 375                 380

Thr Ile Cys Ile Ile Ile Asn Thr Val Phe Leu Ala Met Glu His His
385                 390                 395                 400

Lys Met Glu Ala Ser Phe Glu Lys Met Leu Asn Ile Gly Asn Leu Val
                405                 410                 415

Phe Thr Ser Ile Phe Ile Ala Glu Met Cys Leu Lys Ile Ile Ala Leu
            420                 425                 430

Asp Pro Tyr His Tyr Phe Arg Arg Gly Trp Asn Ile Phe Asp Ser Ile
        435                 440                 445

Val Ala Leu Leu Ser Phe Ala Asp Val Met Asn Cys Val Leu Gln Lys
    450                 455                 460

Arg Ser Trp Pro Phe Leu Arg Ser Phe Arg Val Leu Arg Val Phe Lys
465                 470                 475                 480

Leu Ala Lys Ser Trp Pro Thr Leu Asn Thr Leu Ile Lys Ile Ile Gly
                485                 490                 495

Asn Ser Val Gly Ala Leu Gly Ser Leu Thr Val Val Leu Val Ile Val
            500                 505                 510

Ile Phe Ile Phe Ser Val Val Gly Met Gln Leu Phe Gly Arg Ser Phe
        515                 520                 525

Asn Ser Gln Lys Ser Pro Lys Leu Cys Asn Pro Thr Gly Pro Thr Val
    530                 535                 540

Ser Cys Leu Arg His Trp His Met Gly Asp Phe Trp His Ser Phe Leu
545                 550                 555                 560

Val Val Phe Arg Ile Leu Cys Gly Glu Trp Ile Glu Asn Met Trp Glu
                565                 570                 575

Cys Met Gln Glu Ala Asn Ala Ser Ser Ser Leu Cys Val Ile Val Phe
            580                 585                 590

Ile Leu Ile Thr Val Ile Gly Lys Leu Val Val Leu Asn Leu Phe Ile
        595                 600                 605

Ala Leu Leu Leu Asn Ser Phe Ser Asn Glu Glu Arg Asn Gly Asn Leu
    610                 615                 620

Glu Gly Glu Ala Arg Lys Thr Lys Val Gln Leu Ala Leu Asp Arg Phe
625                 630                 635                 640

Arg Arg Ala Phe Cys Phe Val Arg His Thr Leu Glu His Phe Cys His
                645                 650                 655

Lys Trp Cys Arg Lys Gln Asn Leu Pro Gln Gln Lys Glu Val Ala Gly
            660                 665                 670
```

```
Gly Cys Ala Ala Gln Ser Lys Asp Ile Ile Pro Leu Val Met Glu Met
        675                 680                 685

Lys Arg Gly Ser Glu Thr Gln Glu Glu Leu Gly Ile Leu Thr Ser Val
    690                 695                 700

Pro Lys Thr Leu Gly Val Arg His Asp Trp Thr Trp Leu Ala Pro Leu
705                 710                 715                 720

Ala Glu Glu Glu Asp Asp Val Glu Phe Ser Gly Glu Asp Asn Ala Gln
                725                 730                 735

Arg Ile Thr Gln Pro Glu Pro Glu Gln Gln Ala Tyr Glu Leu His Gln
            740                 745                 750

Glu Asn Lys Lys Pro Thr Ser Gln Arg Val Gln Ser Val Glu Ile Asp
        755                 760                 765

Met Phe Ser Glu Asp Glu Pro His Leu Thr Ile Gln Asp Pro Arg Lys
    770                 775                 780

Lys Ser Asp Val Thr Ser Ile Leu Ser Glu Cys Ser Thr Ile Asp Leu
785                 790                 795                 800

Gln Asp Gly Phe Gly Trp Leu Pro Glu Met Val Pro Lys Lys Gln Pro
                805                 810                 815

Glu Arg Cys Leu Pro Lys Gly Phe Gly Cys Cys Phe Pro Cys Cys Ser
            820                 825                 830

Val Asp Lys Arg Lys Pro Pro Trp Val Ile Trp Trp Asn Leu Arg Lys
        835                 840                 845

Thr Cys Tyr Gln Ile Val Lys His Ser Trp Phe Glu Ser Phe Ile Ile
    850                 855                 860

Phe Val Ile Leu Leu Ser Ser Gly Ala Leu Ile Phe Glu Asp Val His
865                 870                 875                 880

Leu Glu Asn Gln Pro Lys Ile Gln Glu Leu Leu Asn Cys Thr Asp Ile
                885                 890                 895

Ile Phe Thr His Ile Phe Ile Leu Glu Met Val Leu Lys Trp Val Ala
            900                 905                 910

Phe Gly Phe Gly Lys Tyr Phe Thr Ser Ala Trp Cys Cys Leu Asp Phe
        915                 920                 925

Ile Ile Val Ile Val Ser Val Thr Thr Leu Ile Asn Leu Met Glu Leu
    930                 935                 940

Lys Ser Phe Arg Thr Leu Arg Ala Leu Arg Pro Leu Arg Ala Leu Ser
945                 950                 955                 960

Gln Phe Glu Gly Met Lys Val Val Val Asn Ala Leu Ile Gly Ala Ile
                965                 970                 975
```

```
Pro Ala Ile Leu Asn Val Leu Leu Val Cys Leu Ile Phe Trp Leu Val
            980                 985                 990

Phe Cys Ile Leu Gly Val Tyr Phe Phe Ser Gly Lys Phe Gly Lys Cys
        995                1000                1005

Ile Asn Gly Thr Asp Ser Val Ile Asn Tyr Thr Ile Ile Thr Asn Lys
   1010                1015                1020

Ser Gln Cys Glu Ser Gly Asn Phe Ser Trp Ile Asn Gln Lys Val Asn
1025                1030                1035                1040

Phe Asp Asn Val Gly Asn Ala Tyr Leu Ala Leu Leu Gln Val Ala Thr
                1045                1050                1055

Phe Lys Gly Trp Met Asp Ile Ile Tyr Ala Ala Val Asp Ser Thr Glu
            1060                1065                1070

Lys Glu Gln Gln Pro Glu Phe Glu Ser Asn Ser Leu Gly Tyr Ile Tyr
        1075                1080                1085

Phe Val Val Phe Ile Ile Phe Gly Ser Phe Phe Thr Leu Asn Leu Phe
   1090                1095                1100

Ile Gly Val Ile Ile Asp Asn Phe Asn Gln Gln Gln Lys Lys Leu Gly
1105                1110                1115                1120

Gly Gln Asp Ile Phe Met Thr Glu Glu Gln Lys Lys Tyr Tyr Asn Ala
                1125                1130                1135

Met Lys Lys Leu Gly Ser Lys Lys Pro Gln Lys Pro Ile Pro Arg Pro
            1140                1145                1150

Leu Asn Lys Cys Gln Gly Leu Val Phe Asp Ile Val Thr Ser Gln Ile
        1155                1160                1165

Phe Asp Ile Ile Ile Ile Ser Leu Ile Ile Leu Asn Met Ile Ser Met
   1170                1175                1180

Met Ala Glu Ser Tyr Asn Gln Pro Lys Ala Met Lys Ser Ile Leu Asp
1185                1190                1195                1200

His Leu Asn Trp Val Phe Val Val Ile Phe Thr Leu Glu Cys Leu Ile
                1205                1210                1215

Lys Ile Phe Ala Leu Arg Gln Tyr Tyr Phe Thr Asn Gly Trp Asn Leu
            1220                1225                1230

Phe Asp Cys Val Val Val Leu Leu Ser Ile Val
        1235                1240
```

<210> 9
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<221> variation
<222> (6)
<223> r = a or g

<220>
<223> Description of Artificial Sequence: rat NaN forward primer no. 1

<400> 9
gacccrtgga attggttgga 20

<210> 10
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: rat NaN forward primer no. 2

<400> 10
aatccctgga attggttgga 20

<210> 11
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: rat NaN forward primer no. 3

<400> 11
gacccgtgga actggttaga 20

<210> 12
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: rat NaN forward primer no. 4

<400> 12
gatctttgga actggcttga 20

<210> 13
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: rat Nan forward primer no. 5

<400> 13
aacatagtgc tggagttcag g 21

<210> 14
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: rat NaN forward primer no. 6

<400> 14
gtggcctttg gattccggag g 21

<210> 15
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: rat NaN reverse primer no. 1

<400> 15
caagaaggcc cagctgaagg tgtc 24

<210> 16
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: rat NaN reverse primer no. 2

<400> 16
gaggaatgcc cacgcaaagg aatc 24

<210> 17
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: rat NaN reverse primer no. 3

<400> 17
aagaagggac cagccaaagt tgtc 24

<210> 18
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: rat NaN reverse primer no. 4, y = c or t, r = a or g, n = a or c or g or t, w = a or t

<400> 18
acytccatrc anwcccacat 20

<210> 19
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: rat NaN reverse primer no. 5, r = a or g

<400> 19
agraartcna gccarcacca 20

<210> 20
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: rat NaN reverse primer no. 6

<400> 20
tctgctgccg agccaggta 19

<210> 21
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: rat NaN reverse primer no. 7

<400> 21
ctgagataac tgaaatcgcc 20

<210> 22
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer, Marathon AP-1

<400> 22
ccatcctaat acgactcact atagggc 27

<210> 23
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer, Marathon AP-2

<400> 23
actcactata gggctcgagc ggc 23

<210> 24
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: mouse NaN forward primer

<400> 24
ccctgctgcg ctcggtgaag aag 23

<210> 25
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: mouse NaN reverse primer

<400> 25
gacaaagtag atcccagagg 20

<210> 26
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: human NaN forward primer

<400> 26
ctcagtagtt ggcatgc 17

<210> 27
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: human NaN reverse primer

<400> 27
ggaaagaagc acgaccacac agtc 24

<210> 28
<211> 94
<212> PRT
<213> Rattus norvegicus

<220>
<223> C-terminal truncated rat NaN

<400> 28
Ala Ala Gly Gln Ala Met Arg Lys Gln Gly Asp Ile Leu Gly Pro Asn
1 5 10 15

Ile His Gln Phe Ser Gln Ser Ser Glu Thr Pro Phe Leu Gly Cys Pro
20 25 30

Gln Gln Arg Thr Cys Val Ser Phe Val Arg Pro Gln Arg Val Leu Arg
35 40 45

Val Pro Trp Phe Pro Ala Trp Arg Thr Val Thr Phe Leu Ser Arg Pro
50 55 60

Arg Ser Ser Glu Ser Ser Ala Trp Leu Gly Leu Val Glu Ser Ser Gly
65 70 75 80

Trp Ser Gly Leu Pro Gly Glu Ser Gly Pro Ser Ser Leu Leu
85 90

<210> 29
<211> 211
<212> DNA
<213> Mus musculus

<400> 29
agtttaatgt tgagtgaatt gtggtggtga tttcccactt gaggcctttg tgttaaagcc 60

caatgtgtgt gtgtgtgtgt gtgtgtgtgt gtgtgtgtgt gtgtgtgtgt gtgtgtggtt 120
gggggtggt ggcagagtct ggtattggta aggtgagagc aatcccagaa cgtccacctg 180
ctcttccatt ttattaatca ggcaggcctc t 211

<210> 30
<211> 242
<212> DNA
<213> Mus musculus

<400> 30
gtaagccact ggctcttaac taaaatgctc gttggcatta gaacatttct gagctggggt 60
ggtggtggtg gtggtggtgg tggtggtggt ggtggtggtg gtggtggtgg tgatggtggt 120
ggtggaggtg gnggtggagg tggtggctgt ggtggtggng gtggtggtgg tggtggangt 180
ggangtggtg gcgtggtggt ggnggtggtg gtggaggtgg tggctgtggt ggtngtggtg 240
gc 242

<210> 31
<211> 200
<212> DNA
<213> Mus musculus

<400> 31
tgtgcatgct tgattcccag ctcctatggt ctgattactc ggtccttagg agcaaggcca 60
gactgtccac cctgacacac acacacacac acacacacac acacacacac acacacacac 120
acagtgtaga gaattacctc attcttggag tttctctgga aaaggaatgt ctcaaagcca 180
agttcacaga gcaacagctg 200

<210> 32
<211> 181
<212> DNA
<213> Mus musculus

<400> 32
tgttagaaac tctaagacaa tgaagcacca tgctggaaat aagagcacaa actctttctt 60
catgcattac ccactgcttg tgctttcacc ttagtgctcg tgctctctct ttctctctct 120
ctctctctct ctctctctct ctctctctct ctctctctct ctctctcttt ttttttttt 180
t 181

<210> 33
<211> 128
<212> DNA
<213> Mus musculus

<400> 33
cacacacaca cacacacaca cacacacaca cacacacaca gagaaacact gtcgcagtca 60
tacatataaa gataaataca tcttaaaaaa agaaccatgt gattgagtta taaaatattc 120
caacttat 128

<210> 34
<211> 200
<212> DNA

<213> Mus musculus

<400> 34
aggtcatttc ctctgcagtg tgcttggcag gaaaaacttc ctggctattc aagtcagtgc 60
cctgcttgat catccatgta tcacacacac acaaaacaaa caaacaaaca aacaaaaccc 120
tggggaagaa ggaagaggtt aagcacatag gcagagagca gccaggctga ctcagagcaa 180
acacctgatc attcttccat 200

<210> 35
<211> 158
<212> DNA
<213> Mus musculus

<400> 35
gtgctgggat caaaggcgtg cgccgccacc acgcccggcc ccttttttatg tttcaaattt 60
acttttatca tgtgcacgtg tgtgggtgcg tgcatgtgtg tgcgtgcgtg tgcgtgtgng 120
tgtgngtgtg tgtgtgtgtg tgtgtgtgtg tgtgtgtg 158

<210> 36
<211> 113
<212> DNA
<213> Mus musculus

<400> 36
cacacacaca cacacacaca cacacacaca cacacacaca cacacacttg 60
catctttgag ttaattggat aggctgagtc ttacaccgga atcatactgt tgc 113

<210> 37
<211> 200
<212> DNA
<213> Mus musculus

<400> 37
ccaatgagag actcttgtct caaaaaagcc atggtgtcca gatcctgagg aataacacct 60
aagaatgtgc tctggcctga aaacacacac acacacacac acacacacac acacacacac 120
agttttattt atttatttaa aaaaatatgt ctctaggcat tgctgaaatg tctcctacag 180
gattaagtca accagagcca 200

<210> 38
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: protein seq.
basis for rat NaN reverse primers

<220>
<221> VARIANT
<222> (3)
<223> Xaa = Val or Asp

FIG. 1A

Nucleotide sequence of rat NaN. Translation initiation begins at position 41 (ATG). Reading frame ends at position 5336 (TGA).

```
   1  ACGGTGCCCT GATCCTCTGT ACCAGGAAGA CAGGGTGAAG ATGGAGGAGA
  51  GGTACTACCC GGTGATCTTC CCGGACGAGC GGAATTTCCG CCCCTTCACT
 101  TCCGACTCTC TGGCTGCCAT AGAGAAGCGG ATTGCTATCC AAAAGGAGAG
 151  GAAGAAGTCC AAAGACAAGG CGGCAGCTGA GCCCCAGCCT CGGCCTCAGC
 201  TTGACCTAAA GGCCTCCAGG AAGTTACCTA AGCTTTATGG TGACATTCCC
 251  CCTGAGCTTG TAGCGAAGCC TCTGGAAGAC CTGGACCCAT TCTACAAAGA
 301  CCATAAGACA TTCATGGTGT TGAACAAGAA GAGAACAATT TATCGCTTCA
 351  GCGCCAAGCG GGCCTTGTTC ATTCTGGGGC CTTTTAATCC CCTCAGAAGC
 401  TTAATGATTC GTATCTCTGT CCATTCAGTC TTTAGCATGT TCATCATCTG
 451  CACGGTGATC ATCAACTGTA TGTTCATGGC GAATTCTATG GAGAGAAGTT
 501  TCGACAACGA CATTCCCGAA TACGTCTTCA TTGGGATTTA TATTTTAGAA
 551  GCTGTGATTA AAATATTGGC AAGAGGCTTC ATTGTGGATG AGTTTTCCTT
 601  CCTCCGAGAT CCGTGGAACT GGCTGGACTT CATTGTCATT GGAACAGCGA
 651  TCGCAACTTG TTTTCCGGGC AGCCAAGTCA ATCTTTCAGC TCTTCGTACC
 701  TTCCGAGTGT TCAGAGCTCT GAAGGCGATT TCAGTTATCT CAGGTCTGAA
 751  GGTCATCGTA GGTGCCCTGC TGCGCTCGGT GAAGAAGCTG GTAGACGTGA
 801  TGGTCCTCAC TCTCTTCTGC CTCAGCATCT TTGCCCTGGT CGGTCAGCAG
 851  CTGTTCATGG GAATTCTGAA CCAGAAGTGT ATTAAGCACA ACTGTGGCCC
 901  CAACCCTGCA TCCAACAAGG ATTGTTTTGA AAAGGAAAAA GATAGCGAAG
 951  ACTTCATAAT GTGTGGTACC TGGCTCGGCA GCAGACCCTG TCCCAATGGT
1001  TCTACGTGCG ATAAAACCAC ATTGAACCCA GACAATAATT ATACAAAGTT
1051  TGACAACTTT GGCTGGTCCT TTCTCGCCAT GTTCCGGGTT ATGACTCAAG
1101  ACTCCTGGGA GAGGCTTTAC CGACAGATCC TGCGGACCTC TGGGATCTAC
1151  TTTGTCTTCT TCTTCGTGGT GGTCATCTTC CTGGGCTCCT TCTACCTGCT
1201  TAACCTAACC CTGGCTGTTG TCACCATGGC TTATGAAGAA CAGAACAGAA
1251  ATGTAGCTGC TGAGACAGAG GCCAAGGAGA AAATGTTTCA GGAAGCCCAG
1301  CAGCTGTTAA GGGAGGAGAA GGAGGCTCTG GTTGCCATGG GAATTGACAG
1351  AAGTTCCCTT AATTCCCTTC AAGCTTCATC CTTTTCCCCG AAGAAGAGGA
1401  AGTTTTTCGG TAGTAAGACA AGAAAGTCCT TCTTTATGAG AGGGTCCAAG
1451  ACGGCCCAAG CCTCAGCGTC TGATTCAGAG GACGATGCCT CTAAAAATCC
1501  ACAGCTCCTT GAGCAGACCA AACGACTGTC CCAGAACTTG CCAGTGGATC
1551  TCTTTGATGA GCACGTGGAC CCCCTCCACA GGCAGAGAGC GCTGAGCGCT
1601  GTCAGTATCT TAACCATCAC CATGCAGGAA CAAGAAAAAT TCCAGGAGCC
1651  TTGTTTCCCA TGTGGGAAAA ATTTGGCCTC TAAGTACCTG GTGTGGGACT
```

FIG. 1B 2/28

```
1701  GTAGCCCTCA ATGGCTGTGC ATAAAGAAGG TCCTGCGGAC CATCATGACG
1751  GATCCCTTTA CTGAGCTGGC CATCACCATC TGCATCATCA TCAATACCGT
1801  TTTCTTAGCC GTGGAGCACC ACAACATGGA TGACAACTTA AAGACCATAC
1851  TGAAAATAGG AAACTGGGTT TTCACGGGAA TTTTCATAGC GGAAATGTGT
1901  CTCAAGATCA TCGCGCTCGA CCCTTACCAC TACTTCCGGC ACGGCTGGAA
1951  TGTTTTTGAC AGCATCGTGG CCCTCCTGAG TCTCGCTGAT GTGCTNTACA
2001  ACACACTGTC TGATAACAAT AGGTCTTTCT TGGCTTCCCT CAGAGTGCTG
2051  AGGGTCTTCA AGTTAGCCAA ATCCTGGCCC ACGTTAAACA CTCTCATTAA
2101  GATCATCGGC CACTCCGTGG GCGCGCTTGG AAACCTGACT GTGGTCCTGA
2151  CTATCGTGGT CTTCATCTTT TCTGTGGTGG GCATGCGGCT CTTCGGCACC
2201  AAGTTTAACA AGACCGCCTA CGCCACCCAG GAGCGGCCCA GGCGGCGCTG
2251  GCACATGGAT AATTTCTACC ACTCCTTCCT GGTGGTGTTC CGCATCCTCT
2301  GTGGGGAATG GATCGAGAAC ATGTGGGGCT GCATGCAGGA TATGGACGGC
2351  TCCCCGTTGT GCATCATTGT CTTTGTCCTG ATAATGGTGA TCGGGAAGCT
2401  TGTGGTGCTT AACCTCTTCA TTGCCTTGCT GCTCAATTCC TTCAGCAATG
2451  AGGAGAAGGA TGGGAGCCTG GAAGGAGAGA CCAGGAAAAC CAAAGTGCAG
2501  CTAGCCCTGG ATCGGTTCCG CCGGGCCTTC TCCTTCATGC TGCACGCTCT
2551  TCAGAGTTTT TGTTGCAAGA AATGCAGGAG GAAAAACTCG CCAAAGCCAA
2601  AAGAGACAAC AGAAAGCTTT GCTGGTGAGA ATAAAGACTC AATCCTCCCG
2651  GATGCGAGGC CCTGGAAGGA GTATGATACA GACATGGCTT TGTACACTGG
2701  ACAGGCCGGG GCTCCGCTGG CCCCACTCGC AGAGGTAGAG GACGATGTGG
2751  AATATTGTGG TGAAGGCGGT GCCCTACCCA CCTCACAACA TAGTGCTGGA
2801  GTTCAGGCCG GTGACCTCCC TCCAGAGACC AAGCAGCTCA CTAGCCCGGA
2851  TGACCAAGGG GTTGAAATGG AAGTATTTTC TGAAGAAGAT CTGCATTTAA
2901  GCATACAGAG TCCTCGAAAG AAGTCTGACG CAGTGAGCAT GCTCTCGGAA
2951  TGCAGCACAA TTGACCTGAA TGATATCTTT AGAAATTTAC AGAAAACAGT
3001  TTCCCCCAAA AAGCAGCCAG ATAGATGCTT TCCCAAGGGC CTTAGTTGTC
3051  ACTTTCTATG CCACAAAACA GACAAGAGAA AGTCCCCCTG GGTCCTGTGG
3101  TGGAACATTC GGAAAACCTG CTACCAAATC GTGAAGCACA GCTGGTTTGA
3151  GAGTTTCATA ATCTTTGTTA TTCTGCTGAG CAGTGGAGCG CTGATATTTG
3201  AAGATGTCAA TCTCCCCAGC CGGCCCCAAG TTGAGAAATT ACTAAGGTGT
3251  ACCGATAATA TTTTCACATT TATTTTCCTC CTGGAAATGA TCCTGAAGTG
3301  GGTGGCCTTT GGATTCCGGA GGTATTTCAC CAGTGCCTGG TGCTGGCTTG
3351  ATTTCCTCAT TGTGGTGGTG TCTGTGCTCA GTCTCATGAA TCTACCAAGC
3401  TTGAAGTCCT TCCGGACTCT GCGGGCCCTG AGACCTCTGC GGGCGCTGTC
3451  CCAGTTTGAA GGAATGAAGG TTGTCGTCTA CGCCCTGATC AGCGCCATAC
3501  CTGCCATTCT CAATGTCTTG CTGGTCTGCC TCATTTTCTG GCTCGTATTT
3551  TGTATCTTGG GAGTAAATTT ATTTTCTGGG AAGTTTGGAA GGTGCATTAA
3601  CGGGACAGAC ATAAATATGT ATTTGGATTT TACCGAAGTT CCGAACCGAA
```

FIG. 1C


```
3651  GCCAATGTAA CATTAGTAAT TACTCGTGGA AGGTCCCGCA GGTCAACTTT
3701  GACAACGTGG GGAATGCCTA TCTCGCCCTG CTGCAAGTGG CAACCTATAA
3751  GGGCTGGCTG GAAATCATGA ATGCTGCTGT CGATTCCAGA GAGAAAGACG
3801  AGCAGCCGGA CTTTGAGGCG AACCTCTACG CGTATCTCTA CTTTGTGGTT
3851  TTTATCATCT TCGGCTCCTT CTTTACCCTG AACCTCTTTA TCGGTGTTAT
3901  TATTGACAAC TTCAATCAGC AGCAGAAAAA GTTAGGTGGC CAAGACATTT
3951  TTATGACAGA AGAACAGAAG AAATATTACA ATGCAATGAA AAAGTTAGGA
4001  ACCAAGAAAC CTCAAAAGCC CATCCCAAGG CCCCTGAACA ANTGTCAAGC
4051  CTTTGTGTTC GACCTGGTCA CAAGCCATGT CTTTGACGTC ATCATTCTGG
4101  GTCTTATTGT CTTAAATATG ATTATCATGA TGGCTGAATC TGCCGACCAG
4151  CCCAAAGATG TGAAGAAAAC CTTTGATATC CTCAACATAG CCTTCGTGGT
4201  CATCTTTACC ATAGAGTGTC TCATCAAAGT CTTTGCTTTG AGGCAACACT
4251  ACTTCACCAA TGGCTGGAAC TTATTTGATT GTGTGGTCGT GGTTCTTTCT
4301  ATCATTAGTA CCCTGGTTTC CCGCTTGGAG GACAGTGACA TTTCTTTCCC
4351  GCCCACGCTC TTCAGAGTCG TCCGCTTGGC TCGGATTGGT CGAATCCTCA
4401  GGCTGGTCCG GGCTGCCCGG GGAATCAGGA CCCTCCTCTT TGCTTTGATG
4451  ATGTCTCTCC CCTCTCTCTT CAACATCGGT CTGCTGCTCT TCCTGGTGAT
4501  GTTCATTTAC GCCATCTTTG GGATGAGCTG GTTTTCCAAA GTGAAGAAGG
4551  GCTCCGGGAT CGACGACATC TTCAACTTCG AGACCTTTAC GGGCAGCATG
4601  CTGTGCCTCT TCCAGATAAC CACTTCGGCT GGCTGGGATA CCCTCCTCAA
4651  CCCCATGCTG GAGGCAAAAG AACACTGCAA CTCCTCCTCC CAAGACAGCT
4701  GTCAGCAGCC GCAGATAGCC GTCGTCTACT TCGTCAGTTA CATCATCATC
4751  TCCTTCCTCA TCGTGGTCAA CATGTACATC GCTGTGATCC TCGAGAACTT
4801  CAACACAGCC ACGGAGGAGA GCGAGGACCC TCTGGGAGAG GACGACTTTG
4851  AAATCTTCTA TGAGGTCTGG GAGAAGTTTG ACCCCGAGGC GTCGCAGTTC
4901  ATCCAGTATT CGGCCCTCTC TGACTTTGCG GACGCCCTGC CGGAGCCGTT
4951  GCGTGTGGCC AAGCCGAATA AGTTTCAGTT TCTAGTGATG GACTTGCCCA
5001  TGGTGATGGG CGACCGCCTC CATTGCATGG ATGTTCTCTT TGCTTTCACT
5051  ACCAGGGTCC TCGGGGACTC CAGCGGCTTG GATACCATGA AAACCATGAT
5101  GGAGGAGAAG TTTATGGAGG CCAACCCTTT TAAGAAGCTC TACGAGCCCA
5151  TAGTCACCAC CACCAAGAGG AAGGAGGAGG AGCAAGGCGC CGCCGTCATC
5201  CAGAGGGCCT ACCGGAAACA CATGGAGAAG ATGGTCAAAC TGAGGCTGAA
5251  GGACAGGTCA AGTTCATCGC ACCAGGTGTT TTGCAATGGA GACTTGTCCA
5301  GCTTGGATGT GGCCAAGGTC AAGGTTCACA ATGACTGAAC CCTCATCTCC
5351  ACCCCTACCT CACTGCCTCA CAGCTTAGCC TCCAGCCTCT GGCGAGCAGG
5401  CGGCAGACTC ACTGAACACA GGCCGTTCGA TCTGTGTTTT TGGCTGAACG
5451  AGGTGACAGG TTGGCGTCCA TTTTTAAATG ACTCTTGGAA AGATTTCATG
5501  TAGAGAGATG TTAGAAGGGA CTGCAAAGGA CACCGACCAT AACGGAAGGC
5551  CTGGAGGACA GTCCAACTTA CATAAAGATG AGAAACAAGA AGGAAAGATC
```

FIG. 1D

```
5601  CCAGGAAAAC TTCAGATTGT GTTCTCAGTA CATTCCCCAA TGTGTCTGTT
5651  CGGTGTTTTG AGTATGTGAC CTGCCACATG TAGCTCTTTT TTGCATGTAC
5701  GTCAAAACCC TGCAGTAAGT TAATAGCTTG CTACGGGTGT TCCTACCAGC
5751  ATCACAGAAT TGGGTGTATG ACTCAAACCT AAAAGCATGA CTCTGACTTG
5801  TCAGTCAGCA CCCCGACTTT CAGACGCTCC AATCTCTGTC CCAGGTGTCT
5851  AACGAATAAA TAGGTAAAAG AAAAA
```

FIG. 2A 5/28

Predicted amino acid sequence of rat NaN (1765 a.a).

```
1   MEERYYPVIF PDERNFRPFT SDSLAAIEKR IAIQKERKKS KDKAAAEPQP

51  RPQLDLKASR KLPKLYGDIP PELVAKPLED LDPFYKDHKT FMVLNKKRTI
                                                DI-S1
101 YRFSAKRALF ILGPFNPLRS LMIRISVHSV FSMFIICTVI INCMFMANSM
                  DI-S2                                DI-S3
151 ERSFDNDIPE YVFIGIYILE AVIKILARGF IVDEFSFLRD PWNWLDFIVI
                                    DI-S4
201 GTAIATCFPG SQVNLSALRT FRVFRALKAI SVISGLKVIV GALLRSVKKL
                DI-S5
251 VDVMVLTLFC LSIFALVGQQ LFMGILNQKC IKHNCGPNPA SNKDCFEKEK
                                                DI-SS1
301 DSEDFIMCGT WLGSRPCPNG STCDKTTLNP DNNYTKFDNF GWSFLAMFRV
      DI-SS2                              DI-S6
351 MTQDSWERLY RQILRTSGIY FVFFFVVVIF LGSFYLLNLT LAVVTMAYEE

401 QNRNVAAETE AKEKMFQEAQ QLLREEKEAL VAMGIDRSSL NSLQASSFSP

451 KKRKFFGSKT RKSFFMRGSK TAQASASDSE DDASKNPQLL EQTKRLSQNL

501 PVDLFDEHVD PLHRQRALSA VSILTITMQE QEKFQEPCFP CGKNLASKYL
                                    DII-S1
551 VWDCSPQWLC IKKVLRTIMT DPFTELAITI CIIINTVFLA VEHHNMDDNL
                DII-S2                               DII-S3
601 KTILKIGNWV FTGIFIAEMC LKIIALDPYH YFRHGWNVFD SIVALLSLAD
                                    DII-S4
651 VLYNTLSDNN RSFLASLRVL RVFKLAKSWP TLNTLIKIIG HSVGALGNLT
          DII-S5                                   DII-SS1
701 VVLTIVVFIF SVVGMRLFGT KFNKTAYATQ ERPRRRWHMD NFYHSFLVVF
      DII-SS2                                DII-S6
751 RILCGEWIEN MWGCMQDMDG SPLCIIVFVL IMVIGKLVVL NLFIALLLNS
```

FIG. 2B 6/28

801 ESNEEKDGSL EGETRKTKVQ LALDRFRRAF SFMLHALQSF CCKKCRRKNS

851 PKPKETTESF AGENKDSILP DARPWKEYDT DMALYTGQAG APLAPLAEVE

901 DDVEYCGEGG ALPTSQHSAG VQAGDLPPET KQLTSPDDQG VEMEVFSEED

951 LHLSIQSPRK KSDAVSMLSE CSTIDLNDIF RNLQKTVSPK KQPDRCFPKG

DIII-S1

1001 LSCHFLCHKT DKRKSPWVLW WNIRKTCYQI VKHSWFESFI IFVILLSSGA

DIII-S2

1051 LIFEDVNLPS RPQVEKLLRC TDNIFTFIFL LEMILKWVAF GFRRYFTSAW

DIII-S3 DIII-S4

1101 CWLDFLIVVV SVLSLMNLPS LKSFRTLRAL RPLRALSQFE GMKVVVYALI

DIII-S5

1151 SAIPAILNVL LVCLIFWLVF CILGVNLFSG KFGRCINGTD INMYLDFTEV

DIII-SS1 DIII-SS2

1201 PNRSQCNISN YSWKVPQVNF DNVGNAYLAL LQVATYKGWL EIMNAAVDSR

DIII-S6

1251 EKDEQPDFEA NLYAYLYFVV FIIFGSFFTL NLFIGVIIDN FNQQQKKLGG

DIV-S1

1301 QDIFMTEEQK KYYNAMKKLG TKKPQKPIPR PLNRCQAFVF DLVTSHVFDV

1351 IILGLIVLNM IIMMAESADQ PKDVKKTFDI LNIAFVVIFT IECLIKVFAL

DIV-S4

1401 RQHYFTNGWN LFDCVVVVLS IISTLVSRLE DSDISFPPTL FRVVRLARIG

DIV-S5

1451 RILRLVRAAR GIRTLLFALM MSLPSLFNIG LLLFLVMFIY AIFGMSWFSK

DIV-SS1 DIV-SS2

1501 VKKGSGIDDI FNFETFTGSM LCLFQITTSA GWDTLLNPML EAKEHCNSSS

DIV-S6

1551 QDSCQQPQIA VVYFVSYIII SFLIVVNMYI AVILENFNTA TEESEDPLGE

1601 DDFEIFYEVW EKFDPEASQF IQYSALSDFA DALPEPLRVA KPNKFQFLVM

1651 DLPMVMGDRL HCMDVLFAFT KRVLGDSSGL DTMKTMMEEK FMEANPFKKL

FIG. 2C

1701 YEPIVTTTKR KEEEQGAAVI QRAYRKHMEK MVKLRLKDRS SSSHQVFCNG

1751 DLSSLDVAKV KVHND*

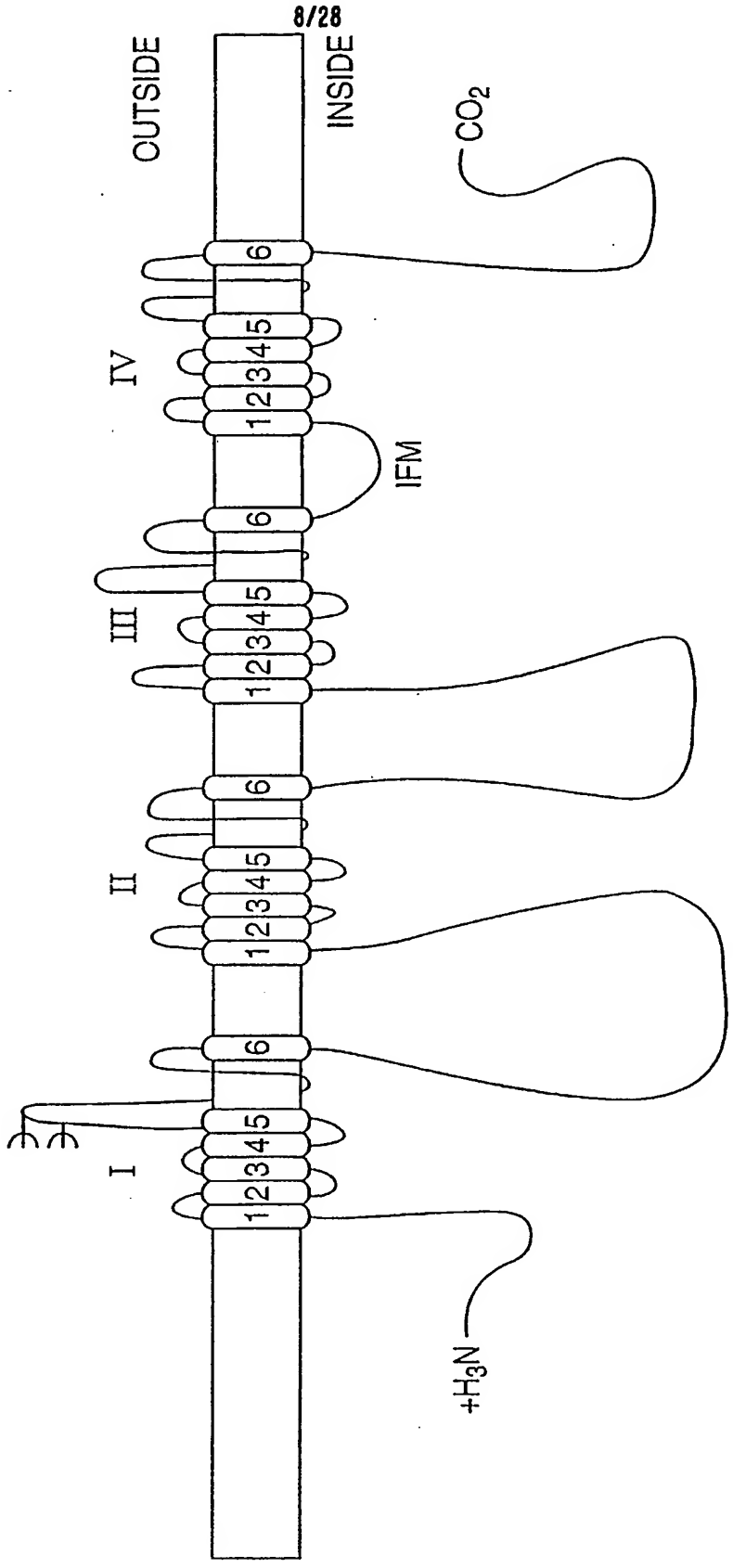

FIG. 3
VOLTAGE-GATED SODIUM CHANNEL α SUBUNIT

FIG. 4

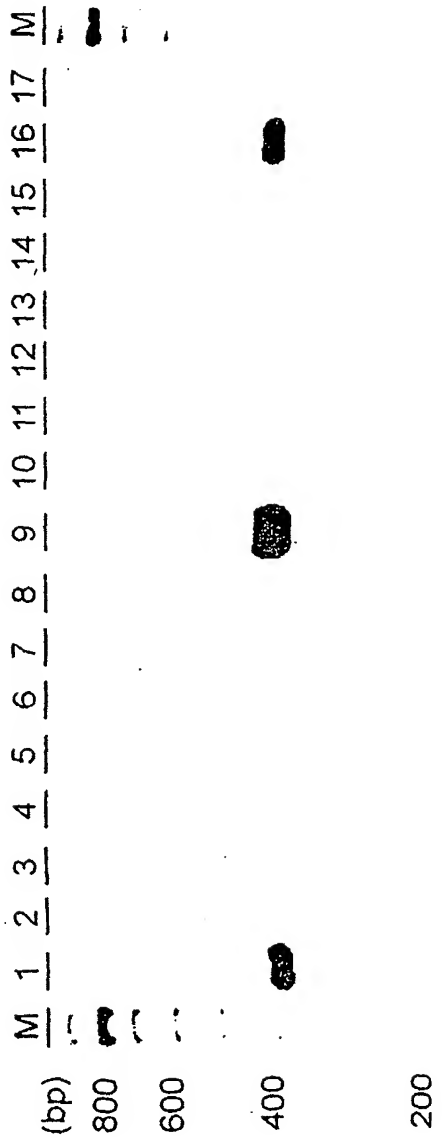

FIG. 5

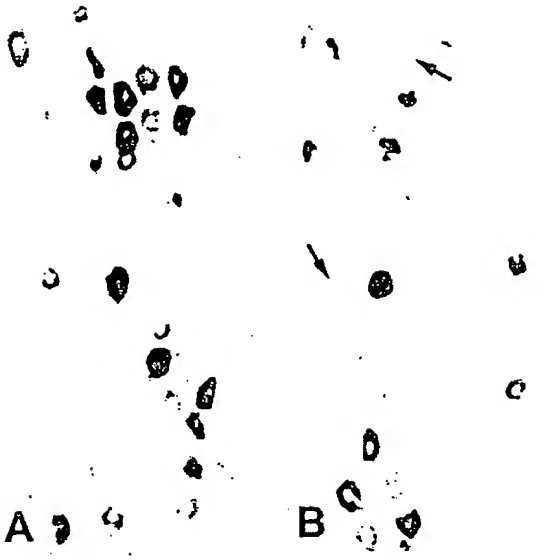

FIG. 6

RESTRICTION ENZYME ANALYSIS OF α-SUBUNIT PCR PRODUCTS FROM DOMAIN I USING THE FOLLOWING PRIMERS: NACHD1A.1-4 (FORWARD PRIMERS) AND NAAGEN.REV1-3 (REVERSE PRIMERS).

Generic Primer pair	F1 R.1	F2 R1	F1 R1	F1/F3 R1	F1 R1	F1/F3 R1	F1 R1	F2 R2	F4 R3	F2 R3
	αI 558 bp	αII 561 bp	αIII 561 bp	αVI 507 bp	αrPN1 501 bp	αrH1 518 bp	αμ1 602 bp	αSNS 479 bp	αNaG 501 bp	αNaN 468 bp
EcoR V	+ 152, 406	-	-	-	-	-	-	-	-	-
EcoN I	-	+ 204, 357	-	-	-	-	-	-	-	-
Ava I	-	-	+ 279, 282	-	-	-	-	-	-	-
Sph I	-	-	-	+ 126, 381	-	-	-	-	-	-
Bam H I	-	-	-	-	+ 134, 367	-	-	+ 126, 353	-	-
Acc I	-	-	-	-	-	+ 173, 345	-	-	-	+ 185, 283
Ngo M I	-	-	-	-	-	-	+ 200, 402	-	+ 95, 406	-
Afl II	-	-	-	-	-	-	-	+ 224, 255	-	-
Xba I	-	-	-	-	-	-	-	-	+ 165, 336	-
EcoR I	-	-	-	-	-	-	-	-	-	+ 214, 254

FIG. 7A-1

Sequence of th mouse NaN cDNA.

```
1     TCTGAGCCAA GGGTGAAGAT GGAGGAGAGG TACTATCCAG TGATCTTCCC AGACGAGAGG
61    AATTTCCGCC CCTTCACTTT CGACTCTTTG GCTGCAATAG AGAAGCGGAT CACCATCCAA
121   AAGGAGAAGA AGAAATCCAA AGACAAGGCA GCAACTGAGC CCCAGCCTCG GCCTCAGCTC
181   GACCTAAAGG CCTCCAGGAA GTTACCTAAG CTCTATGGCG ACGTTCCCCC TGACCTTATA
241   GCGAAGCCCC TGGAAGATCT GGACCCATTT TACAAAGACC ATAAGACATT CATGGTATTG
301   AACAAGAAGA GAACAATCTA TCGCTTCAGC GCCAAGAGGG CCTTGTTCAT TCTGGGGCCT
361   TTTAATCCCA TCAGAAGCTT CATGATTCGC ATCTCTGTCC ATTCAGTCTT CAGCATGTTC
421   ATTATCTGCA CAGTGATCAT CAACTGTATG TTCATGGCTA ATAATTCTTC TGTGGACAGT
481   CGTCCTAGCA GTAACATTCC CGAATACGTC TTCATTGGGA TTTATGTTTT AGAAGCTGTG
541   ATTAAAATAT TGGCAAGAGG CTTCATTGTG GATGAGTTTT CCTACCTCCG AGATCCTTGG
601   AACTGGCTGG ACTTCATTGT CATCGGAACA GCGATAGCGC CTTGTTTTCT CGGTAACAAA
661   GTCAATAATC TTTCCACTCT ACGTACCTTC CGAGTGTTGA GAGCTCTGAA AGCCATTTCT
721   GTAATCTCAG GTCTGAAGGT CATCGTGGGT GCCCTGCTGC GCTCCGTGAA GAAGCTAGTG
781   GACGTGATGG TCCTCACTCT CTTTTGCCTC AGCATCTTTG CCCTGGTTGG TCAGCAGCTC
841   TTCATGGGAA TTCTGAGCCA GAAATGTATT AAGGACGACT GTGGCCCTAA CGCTTTTTCC
901   AACAAGGATT GCTTTGTAAA AGAAAATGAT AGCGAGGACT TCATAATGTG TGGCAACTGG
961   CTCGGCAGAA GATCCTGCCC CGATGGTTCC ACGTGCAATA AAACCACATT TAACCCAGAT
1021  TATAATTATA CAAACTTTGA CAGCTTTGGC TGGTCTTTTC TCGCCATGTT CCGGGTTATG
1081  ACTCAAGACT CCTGGGAGAA GCTTTATCGA CAGATCCTTC GCACCTCCGG GATCTACTTT
1141  GTCTTCTTCT TCGTGGTCGT CATCTTCCTG GGCTCTTTCT ACCTGCTTAA CTTAACCCTG
1201  GCTGTCGTCA CCATGGCTTA CGAGGAACAG AACAGAAATG TCGCTGCCGA GACAGAGGCC
1261  AAGGAGAAGA TGTTTCAGGA AGCCCAGCAG CTGTTGAGGG AGGAAAAGGA GGCTCTGGTT
1321  GCCATGGGAA TTGACAGAAC TTCCCTTAAT TCCCTCCAAG CTTCGTCCTT TTCCCCAAAG
1381  AAGAGGAAGT TTTTTGGCAG TAAGACAAGA AAGTCCTTCT TTATGAGAGG GTCCAAGACA
1441  GCCCGAGCCT CAGCGTCCGA TTCAGAGGAC GATGCCTCTA AAAACCCACA ACTCCTTGAG
1501  CAAACAAAAC GACTATCCCA GAACTTGCCC GTAGAACTCT TTGATGAGCA CGTGGACCCC
1561  CTCCATAGGC AGAGAGCGCT GAGTGCCGTC AGTATCTTAA CCATCACCAT GCAGGAACAA
1621  GAAAAATCCC AGGAGCCTTG TTTCCCGTGT GGGAAAAACT TGGCATCCAA GTACCTGGTG
1681  TGGGAATGTA GCCCTCCGTG GCTGTGCATA AAGAAGGTCC TGCAGACTAT CATGACAGAC
1741  CCCTTCACTG AGCTGGCCAT CACCATCTGC ATCATCGTCA ATACTGTCTT CTTGGCCATG
1801  GAACACCACA ATATGGATAA CTCTTTAAAA GACATACTGA AAATAGGAAA CTGGGTTTTC
1861  ACTGGAATTT TCATAGCGGA AATGTGTCTC AAGATCATTG CGCTAGACCC TTACCACTAC
1921  TTCCGGCACG GCTGGAACAT CTTTGACAGC ATTGTGGCCC TTGTGAGTCT CGCTGACGTG
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FIG. 7A-2

```
1981  CTCTTCCACA AACTGTCTAA AAACCTCTCC TTCTTGGCTT CCCTCAGAGT GCTGAGGGTC
2041  TTCAAGTTAG CCAAATCCTG GCCCACATTA AACACTCTCA TTAAGATCAT CGGCCACTCC
2101  GTGGGTGCGC TCGGAAACCT GACTGTGGTC CTAACGATCG TGGTCTTCAT CTTTTCCGTG
2161  GTTGGCATGC GGCTCTTTGG TGCCAAGTTT AACAAGACTT GCTCCACCTC TCCGGAGTCC
2221  CTCCGGCGCT GGCACATGGG TGATTTCTAC CATTCCTTCC TGGTGGTGTT CCGCATCCTC
2281  TGTGGGGAGT GGATCGAGAA CATGTGGGAA TGCATGCAGG AGATGGAAGG CTCCCCGCTG
2341  TGTGTCATCG TCTTTGTGCT GATCATGGTG GTCGGGAAGC TCGTGGTGCT TAACCTCTTC
2401  ATTGCCTTGC TGCTCAATTC CTTCAGCAAT GAGGAAAAGG ATGGGAACCC AGAAGGAGAG
2461  ACCAGGAAAA CCAAAGTGCA GCTAGCCCTG GATCGGTTCA GCCGAGCGTT CTACTTCATG
2521  GCGCGCGCTC TTCAGAATTT CTGTTGCAAG AGATGCAGGA GGCAAAACTC GCCAAAGCCA
2581  AATGAGGCAA CAGAAAGCTT TGCTGGTGAG AGTAGAGACA CAGCCACCCT GGATACAAGG
2641  TCCTGGAAGG AGTATGATTC AGAAATGACT CTGTACACTG GGCAGGCCGG GGCTCCACTG
2701  GCCCCACTGG CAAAAGAAGA GGACGATATG GAATGTTGTG GTGAATGTGA TGCCTCACCT
2761  ACCTCACAGC CTAGTGAGGA AGCTCAGGCC TGTGACCTCC CTCTGAAGAC CAAGCGGCTC
2821  CCCAGCCCAG ATGACCACGG GGTTGAAATG GAAGTGTTTT CCGAAGAAGA TCCGAATTTA
2881  ACCATACAGA GTGCTCGAAA GAAGTCTGAT GCGGCAAGCA TGCTCTCAGA ATGCAGCACA
2941  ATAGACCTGA ATGATATCTT TAGAAATTTA CAGAAAACAG TTTCCCCCCA AAAGCAACCA
3001  GATCGATGCT TTCCCAAGGG CCTCAGTTGT ATCTTTCTAT GTTGCAAAAC AATCAAAAAA
3061  AAGTCCCCCT GGGTCCTGTG GTGGAATCTT CGGAAAACCT GCTACCAAAT CGTGAAGCAT
3121  AGCTGGTTTG AGAGCTTCAT AATTTTTGTC ATCCTGCTGA GCAGCGGAGC ACTGATATTC
3181  GAAGATGTCA ATCTTCCCAG CCGGCCCCAA GTTGAAAAAT TACTGAAGTG TACCGATAAT
3241  ATTTTCACAT TTATTTTTCT CCTGGAAATG ATTTTGAAGT GGGTGGCCTT TGGATTCCGG
3301  AAGTATTTCA CCAGTGCCTG GTGCTGGCTC GATTTCCTCA TTGTGGTGGT GTCTGTGCTC
3361  AGCCTCACGA ACTTACCAAA CTTGAAGTCC TTCCGGAATC TGCGAGCGCT GAGACCTCTG
3421  CGGGCACTGT CTCAGTTTGA AGGAATGAAG GTTGTTGTCA ATGCCCTCAT GAGTGCCATA
3481  CCTGCCATCC TCAATGTCTT GCTGGTCTGC CTCATTTTCT GGCTCATATT TTGTATCCTG
3541  GGAGTAAATT TTTTTTCTGG GAAGTTTGGA AGATGCATTA ATGGAACAGA CATAAATAAA
3601  TATTTCAACG CTTCCAATGT TCCAAACCAA AGCCAATGTT TAGTTAGTAA TTACACGTGG
3661  AAAGTCCCGA ATGTCAACTT TGACAACGTG GGGAATGCCT ACCTTGCCCT GCTGCAAGTG
3721  GCGACCTATA AGGGCTGGCT GGACATTATG AATGCAGCTG TTGATTCCAG AGGGAAAGAT
3781  GAGCAGCCGG CCTTTGAGGC GAATCTATAC GCATACCTTT ACTTCGTGGT TTTTATCATC
3841  TTCGGCTCAT TCTTTACCCT GAACCTCTTT ATCGGTGTTA TTATTGACAA CTTCAATCAG
3901  CAGCAGAAAA AGTTAGGTGG CCAAGACATT TTTATGACAG AAGAACAGAA GAAATATTAC
3961  AATGCAATGA AAAAGTTAGG AACCAAGAAG CCTCAAAAGC CCATCCCAAG GCCCCTGAAC
4021  AAATGTCAAG CCTTCGTGTT CGATTTGGTC ACAAGCCAGG TCTTTGACGT CATCATTCTG
4081  GGTCTTATTG TCACAAACAT GATTATCATG ATGGCTGAAT CTGAAGGCCA GCCCAACGAA
4141  GTGAAGAAAA TCTTTGATAT TCTCAACATA GTCTTCGTGG TCATCTTTAC CGTAGAGTGT
4201  CTCATCAAAG TCTTTGCTTT GAGGCAACAC TACTTCACCA ATGGCTGGAA CTTATTTGAT
```

FIG. 7A-3

```
4261  TGTGTGGTCG TGGTTCTTTC CATCATTAGT ACCTTGGTTT CTGGCTTGGA GAACAGCAAC
4321  GTCTTCCCGC CCACACTCTT CAGGATTGTC CGCTTGGCTC GGATCGGTCG AATCCTCAGA
4381  CTGGTCCGGG CGGCTCGAGG AATCAGGACA CTCCTTTTCG CGTTGATGAT GTCTCTCCCC
4441  TCTCTCTTCA ACATTGGTCT GCTTCTCTTT CTGGTGATGT TCATTTATGC CATCTTTGGG
4501  ATGAACTGGT TTTCCAAAGT GAAGAGAGGC TCTGGGATTG ATGACATCTT CAACTTTGAC
4561  ACTTTCTCGG GCAGCATGCT CTGCCTCTTC CAGATAACCA CTTCAGCCGG CTGGGATGCT
4621  CTCCTCAACC CCATGCTGGA ATCAAAAGCC TCTTGCAATT CCTCCTCCCA AGAGAGCTGT
4681  CAGCAGCCGC AGATAGCCAT AGTCTACTTC GTCAGCTACA TCATCATCTC CTTTCTCATT
4741  GTGGTTAACA TGTACATAGC TGTGATTCTA GAGAACTTCA ACACAGCCAC AGAGGAGAGC
4801  GAGGACCCCC TGGGCGAAGA CGACTTTGAG ATCTTCTATG AGATCTGGGA GAAGTTTGAC
4861  CCCGAAGCAA CACAGTTCAT CCAGTACTCA TCCCTCTCTG ACTTCGCCGA CGCCCTGCCC
4921  GAGCCGTTGC GTGTGGCCAA GCCCAACAGG TTTCAGTTTC TCATGATGGA CTTGCCCATG
4981  GTGATGGGTG ATCGCCTCCA TTGCATGGAT GTTCTCTTTG CTTTCACCAC CAGGGTCCTC
5041  GGGAACTCCA GCGGCTTGGA TACCATGAAA GCCATGATGG AGGAGAAGTT CATGGAGGCC
5101  AATCCTTTCA AGAAGTTGTA CGAGCCCATT GTCACCACCA CAAAGAGGAA GGAGGAGGAG
5161  GAATGTGCCG CTGTCATCCA GAGGGCCTAC CGGAGACACA TGGAGAAGAT GATCAAGCTG
5221  AAGCTGAAAG GCAGGTCAAG TTCATCGCTC CAGGTGTTTT GCAATGGAGA CTTGTCTAGC
5281  TTGGATGTGC GCAAGATCAA GGTTCATTGT GACTGAAACC CCCACCTGCA CGCCTACCTC
5341  ACAGCCTCAC AGCTCAGCCC CCAGCCTCTG GCGAACAAGC GGCGGACTCA CCGAACAGGC
5401  CGTTCAACTT GTTTTTTTGG GTGAAAGAGG TGATAGGTTG GTGTCCATTT TTAAATGATT
5461  CTTGGAAAGA TTGAACGTCG GAACATGTTA GAAAGGACTG CCAAGGACAT CCACAGTAAC
5521  GGAAGGCCTG AAGGACAGTT CAAATTATGT AAAGAAACGA GAAGGAAAGG TCACATGTCT
5581  GTTCAGTTTT AAGTATGTGA CCTGCCACAT GTAGCTCCTT TGCATGTTAA GTGAGAAGTC
5641  AAAACCCTGC CATAAGTAAA TAGCTTTGTT GCAGGTGTTT CTACCAGTGC TGCGGATTTG
5701  GGTGTATGGC TCAAACCTGA AAGCATGACT CTGACTTGTC AGCACCCCAA CTTTCAGAAG
5761  CTCTGATCTC TGTCCTAGGT GTTTGACAAA TAAATACATA AAANAAAAAA AAAAAAAAAA
5821  AA
```

FIG. 7B-1

Protein sequence of mNaN

Molecular Weight 201451.00 Daltons

1765 Amino Acids

198 Strongly Basic(+) Amino Acids (K,R)

177 Strongly Acidic(-) Amino Acids (D,E)

712 Hydrophobic Amino Acids (A,I,L,F,W,V)

453 Polar Amino Acids (N,C,Q,S,T,Y)

8.260 Isolectric Point

22.540 Charge at PH 7.0

```
1    MEERYYPVIF PDERNFRPFT FDSLAAIEKR ITIQKEKKKS KDKAATEPQP RPQLDLKASR
21   KLPKLYGDVP PDLIAKPLED LDPFYKDHKT FMVLNKKRTI YRFSAKRALF ILGPFNPIRS
121  FMIRISVHSV FSMFIICTVI INCMFMANNS SVDSRPSSNI PEYVFIGIYV LEAVIKILAR
                DI-S1                                        DI-S2
181  GFIVDEFSYL RDPWNWLDFI VIGTAIAPCF LGNKVNNLST LRTFRVLRAL KAISVISGLK
                              DI-S3                    DI-S4
241  VIVGALLRSV KKLVDVMVLT LFCLSIFALV GQQLFMGILS QKCIKDDCGP NAPSNKDCFV
                              DI-S5
301  KENDSEDFIM CGNWLGRRSC PDGSTCNKTT FNPDYNYTNF DSFGHSFLAM FRVMTQDSWE
                                                 DI-SS1      DI-SS2
361  KLYRQILRTS GIYFVFFFVV VIFLGSFYLL NLTLAVVTMA YEEQNRNVAA ETEAKEKMFQ
                              DI-S6
421  EAQQLLREEK EALVAMGIDR TSLNSLQASS FSPKKRKFFG SKTRKSFFMR GSKTARASAS
481  DSEDDASKNP QLLEQTKRLS QNLPVELFDE HVDPLHRQRA LSAVSILTIT MQEQEKSQEP
541  CFFCGKNLAS KYLVWECSPP WLCIKKVLQT IMTDPFTELA ITICIIVNTV FLAMEHHNMD
                                            DII-S1
601  NSLKDILKIG NWVFTGIFIA EMCLKIIALD PYHYFRHGWN IFDSIVALVS LADVLFHKLS
                DII-S2                              DII-S3
661  KNLSFLASLR VLRVFKLAKS WPTLNTLIKI IGHSVGALGN LTVVLTIVVF IFSVVGMRLF
                DII-S4                                   DII-S5
721  GAKFNKTCST SPESLRRWHM GDFYHSFLVV FRILCGEWIE NMWECMQEMS GSPLCVIVFV
                              DII-SS1    DII-SS2
```

FIG. 7B-2

781 LIMVVGKLVV LNLFIALLLN SFSNEEKDGN PEGETRKTKV QLALDRFSRA FYFMARALQN
DII-S6
841 FCCKRCRRQN SPKPNEATES FAGESRDTAT LDTRSWKEYD SEMTLYTGQA GAPLAPLAKE
901 EDDMECCGEC DASPTSQPSE EAQACDLPLK TKRLPSPDDH GVEMEVFSEE DPNLTIQSAR
961 KKSDAASMLS ECSTIDLNDI FRNLQKTVSP QKQPDRCFPK GLSCIFLCCK TIKKKSPWVL
1021 WWNLRKTCYQ IVKHSWFESF IIFVILLSSG ALIFEDVNLP SRPQVEKLLK CTDNIFTFIF
DIII-S1 DIII-S2
1081 LLEMILKWVA FGFRKYFTSA WCWLDFLIVV VSVLSLTNLP NLKSFRNLRA LRPLRALSQF
DIII-S2 DIII-S3 DIII-S4
1141 EGMKVVVNAL MSAIPAILNV LLVCLIFWLI FCILGVNFFS GKFGRCINGT DINKYFNASN
DIII-S5
1201 VPNQSQCLVS NYTWKVPNVN FDNVGNAYLA LLQVATYKGW LDIMNAAVDS RGKDEQPAFE
DIII-SS1 DIII-SS2
1261 ANLYAYLYFV VFIIFGSFFT LNLFIGVIID NFNQQQKKLG GQDIFMTEEQ KKYYNAMKKL
DIII-S6
1321 GTKKPQKPIP RPLNKCQAFV FDLVTSQVFD VIILGLIVTN MIIMMAESEG QPNEVKKIFD
DIV-S1
1381 ILNIVFVVIF TVECLIKVFA LRQHYFTNGW NLFDCVVVVL SIISTLVSGL ENSNVFPPTL
DIV-S2 DIV-S3
1441 FRIVRLARIG RILRLVRAAR GIRTLLFALM MSLPSLFNIG LLLFLVMFIY AIFGMNWFSK
DIV-S4 DIV-S5
1501 VKRGSGIDDI FNFDTFSGSM LCLFQITTSA GWDALLNPML ESKASCNSSS QESCQQPQIA
DIV-SS1 DIV-SS2
1561 IVYFVSYIII SFLIVVNMYI AVILENFNTA TEESEDPLGE DDFEIFYEIW EKFDPEATQF
DIV-S6
1621 IQYSSLSDFA DALPEPLRVA KPNRFQFLMM DLPMVMGDRL HCMDVLFAFT TRVLGNSSGL
1681 DTMKAMMEEK FMFANPFKKL YEPIVTTTKR KEEEECAAVI QRAYRRHMEK MIKLKLKGRS
1741 SSSLQVFCNG DLSSLDVPKI KVHCD.

FIG. 8A-1 Partial Human NaN Nucleotide Sequence

TCCATTGTCATTGGAATAGCGATTGTGTCATATATTCCAGGAATCACCATCAAACTATTGCCCC
TGCGTACCTTCCGTGTGTTCAGAGCTTTGAAAGCAATTTCAGTAGTTTCACGTCTGAAGGTCAT
CGTGGGGGCCTTGCTACGCTCTGTGAAGAAGCTGGTCAACGTGATTATCCTCACCTTCTTTTGC
CTCAGCATCTTTGCCCTGGTAGGTCAGCAGCTCTTCATGGGAAGTCTGAACCTGAAATGCATCT
CGAGGGACTGTAAAAATATCAGTAACCCGGAAGCTTATGACCATTGCTTTGAAAAGAAAGAAA
ATTCACCTGAATTCAAAATGTGTGGCATCTGGATGGGTAACAGTGCCTGTTCCATACAATATGA
ATGTAAGCACACCAAAATTAATCCTGACTATAATTATACGAATTTTGACAACTTTGGCTGGTCT
TTTCTTGCCATGTTCCGGCTGATGACCCAAGATTCCTGGGAGAAGCTTTATCAACAGACCCTGC
GTACTACTGGGCTCTACTCAGTCTTCTTCTTCATTGTGGTCATTTTCCTGGGCTCCTTCTACCTGA
TTAACTTAACCCTGGCTGTTGTTACCATGGCATATGAGGAGCAGAACAAGAATGTAGCTGCAG
AGATAGAGGCCAAGGAAAAGATGTTTCAGGAAGCCCAGCAGCTGTTAAAGGAGGAAAAGGAG
GCTCTGGTTGCCATGGGAATTGACAGAAGTTCACTTACTTCCCTTGAAACATCATATTTTACCC
CAAAAAAGAGAAAGCTCTTTGGTAATAAGAAAAGGAAGTCCTTCTTTTTGAGAGAGTCTGGGA
AAGACCAGCCTCCTGGGTCAGATTCTGATGAAGATTGCCAAAAAAAAGCCACAGCTCCTAGAGC
AAACCAAACGACTGTCCCAGAATCTATCAYTGGACCACTTTGATGAGCATGGAGATCCTCTCCA
AAGGCAGAGAGCACTGAGTGCTGTCAGCATCCTCACCATCACCATGAAGGAACAAGAAAAATC
ACAAGAGCCTTGTCTCCCTTGTGGAGAAAACCTGGCATCCAAGTACCTCGTGTGGAACTGTTGC
CCCCAGTGGCTGTGCGTTAAGAAGGTCCTGAGAACTGTGATGACTGACCCGTTTACTGAGCTGG
CCATCACCATCTGCATCATCATCAACACTGTCTTCTTGGCCATGGAGCATCACAAGATGGAGGC
CAGTTTTGAGAAGATGTTGAATATAGGGAATTTGGTTTTCACTAGCATTTTTATAGCAGAAATG
TGCCTAAAAATCATTGCGCTCGATCCCTACCACTACTTTCGCCGAGGCTGGAACATTTTTGACA
GCATTGTTGCTCTTCTGAGTTTTGCAGATGTAATGAACTGTGTACTTCAAAAGAGAAGCTGGCC
ATTCTTGCGTTCCTTCAGAGTGCTCAGGGTCTTCAAGTTAGCCAAATCCTGGCCAACTTTGAAC
ACACTAATTAAGATAATCGGCAACTCTGTCGGAGCCCTTGGAAGCCTGACTGTGGTCCTGGTCA
TTGTGATCTTTATTTTCTCAGTAGTTGGCATGCAGCTTTTTGGCCGTAGCTTCAATTCCCAAAAG
AGTCCAAAACTCTGTAACCCGACAGGCCCGACAGTCTCATGTTTACGGCACTGGCACATGGGG
GATTTCTGGCACTCCTTCCTAGTGGTATTCCGCATCCTCTGCGGGGAATGGATCGAAAATATGT
GGGAATGTATGCAAGAAGCGAATGCATCATCATCATTGTGTGTTATTGTCTTCATATTGATCAC
GGTGATAGGAAAACTTGTGGTGCTCAACCTCTTCATTGCCTTACTGCTCAATTCCTTTAGCAAT
GAGGAAAGAAATGGAAACTTAGAAGGAGAGGCCAGGAAAACTAAAGTCCAGTTAGCACTGGA
TCGATTCCGCCGGGCTTTTTGTTTTGTGAGACACACTCTTGAGCATTTCTGTCACAAGTGGTGCA
GGAAGCAAAACTTACCACAGCAAAAAGAGGTGGCAGGAGGCTGTGCTGCACAAAGCAAAGAC
ATCATTCCCCTGGTCATGGAGATGAAAAGGGGCTCAGAGACCCAGGAGGAGCTTGGTATACTA
ACCTCTGTACCAAAGACCCTGGGCGTCAGGCATGATTGGACTTGGTTGGCACCACTTGCGGAG
GAGGAAGATGACGTTGAATTTTCTGGTGAAGATAATGCACAGCGCATCACACAACCTGAGCCT
GAACAACAGGCCTATGAGCTCCATCAGGAGAACAAGAAGCCCACGAGCCAGAGAGTTCAAAG
TGTGGAAATTGACATGTTCTCTGAAGATGAGCCTCATCTGACCATACAGGATCCCCGAAAGAA
GTCTGATGTTACCAGTATACTATCAGAATGTAGCACCATTGATCTTCAGGATGGCTTTGGATGG
TTACCTGAGATGGTTCCCAAAAAGCAACCAGAGAGATGTTTGCCCAAAGGCTTTGGTTGCTGCT
TTCCATGCTGTAGCGTGGACAAGAGAAAGCCTCCCTGGGTCATTTGGTGGAACCTGCGGAAAA

FIG. 8A-2

CCTGCTACCAAATAGTGAAACACAGCTGGTTTGAGAGCTTTATTATCTTTGTGATTCTGCTGAG
CAGTGGGGCACTGATATTTGAAGATGTTCACCTTGAGAACCAACCCAAAATCCAAGAATTACT
AAATTGTACTGACATTATTTTTACACATATTTTTATCCTGGAGATGGTACTAAAATGGGTAGCC
TTCGGATTTGGAAAGTATTTCACCAGTGCCTGGTGCTGCCTTGATTTCATCATTGTGATTGTCTC
TGTGACCACCCTCATTAACTTAATGGAATTGAAGTCCTTCCGGACTCTACGAGCACTGAGGCCT
CTTCGTGCGCTGTCCCAGTTTGAAGGAATGAAGGTGGTGGTCAATGCTCTCATAGGTGCCATAC
CTGCCATTCTGAATGTTTTGCTTGTCTGCCTCATTTTCTGGCTCGTATTTTGTATTCTGGGAGTAT
ACTTCTTTTCTGGAAAATTTGGGAAATGCATTAATGGAACAGACTCAGTTATAAATTATACCAT
CATTACAAATAAAAGTCAATGTGAAAGTGGCAATTTCTCTTGGATCAACCAGAAAGTCAACTTT
GACAATGTGGGAAATGCTTACCTCGCTCTGCTGCAAGTGGCAACATTTAAGGGCTGGATGGAT
ATTATATATGCAGCTGTTGATTCCACAGAGAAAGAACAACAGCCAGAGTTTGAGAGCAATTCA
CTCGGTTACATTTACTTCGTAGTCTTTATCATCTTTGGCTCATTCTTCACTCTGAATCTCTTCATT
GGCGTTATCATTGACAACTTCAACCAACAGCAGAAAAAGTTAGGTGGCCAAGACATTTTTATG
ACAGAAGAACAGAAGAAATACTATAATGCAATGAAAAAATTAGGATCCAAAAAACCTCAAAA
ACCCATTCCACGGCCTCTGAACAAATGTCAAGGTCTCGTGTTCGACATAGTCACAAGCCAGATC
TTTGACATCATCATCATAAGTCTCATTATCCTAAACATGATTAGCATGATGGCTGAATCATACA
ACCAACCCAAAGCCATGAAATCCATCCTTGACCATCTCAACTGGGTCTTTGTGGTCATCTTTAC
GTTAGAATGTCTCATCAAAATCTTTGCTTTGAGGCAATACTACTTCACCAATGGCTGGAATTTA
TTTGA

FIG. 8B

Partial Human NaNAmino Acid Sequence

SI VIGIAIVSYI PGITIKLLPL RTFRVFRALK AISVVSRLKV IVGALLRSVK KLVNVIILTF
FCLSIFALVG QQLFMGSLNL KCISRDCKNI SNPEAYDHCF EKKENSPEFK MCGIWMGNSA
CSIQYECKHT KINPDYNYTN FDNFGWSFLA MFRLMTQDSW EKLYQQTLRT TGLYSVFFFI
VVIFLGSFYL INLTLAVVTM AYEEQNKNVA AEIEAKEKMF QEAQQLLKEE KEALVAMGID
RSSLTSLETS YFTPKKRKLF GNKKRKSFFL RESGKDQPPG SDSDEDCQKK PQLLEQTKRL
SQNLSLDHFD EHGDPLQRQR ALSAVSILTI TMKEQEKSQE PCLPCGENLA SKYLVWNCCP
QWLCVKKVLR TVMTDPFTEL AITICIIINT VFLAMEHHKM EASFEKMLNI GNLVFTSIFI
AEMCLKIIAL DPYHYFRRGW NIFDSIVALL SFADVMNCVL QKRSWPFLRS
FRVLRVFKLAKSWPTLNTLI KIIGNSVGAL GSLTVVLVIV IFIFSVVGMQ LFGRSFNSQK
SPKLCNPTGP TVSCLRHWHM GDFWHSFLVV FRILCGEWIE NMWECMQEAN ASSSLCVIVF
ILITVIGKLV VLNLFIALLL NSFSNEERNG NLEGEARKTK VQLALDRFRR AFCFVRHTLE
IIFCHKWCRKQ NLPQQKEVAG GCAAQSKDII PLVMEMKRGS ETQEELGILT SVPKTLGVRH
DWTWLAPLAE EEDDVEFSGE DNAQRITQPE PEQQAYELHQ ENKKPTSQRVQSVEIDMFSE
DEPHLTIQDP RKKSDVTSIL SECSTIDLQD GFGWLPEMVP KKQPERCLPK GFGCCFPCCS
VDKRKPPWVI WWNLRKTCYQ IVKHSWFESF IIFVILLSSG ALIFEDVHLE NQPKIQELLN
CTDIIFTHIF ILEMVLKWVA FGFGKYFTSA WCCLDFIIVI VSVTTLINLM ELKSFRTLRA
LRPLRALSQF EGMKVVVNAL IGAIPAILNV LLVCLIFWLV FCILGVYFFS GKFGKCINGT
DSVINYTIIT NKSQCESGNF SWINQKVNFD NVGNAYLALL QVATFKGWMD IIYAAVDSTE
KEQQPEFESN SLGYIYFVVF IIFGSFFTLN LFIGVIIDNF NQQQKKLGGQ DIFMTEEQKK
YYNAMKKLGS KKPQKPIPRP LNKCQGLVFD IVTSQIFDII IISLIILNMI SMMAESYNQP
KAMKSILDHL NWVFVVIFTL ECLIKIFALR QYYFTNGWNL FDCVVVLLSIV

FIG. 9

NaN immunostaining in DRG neurons

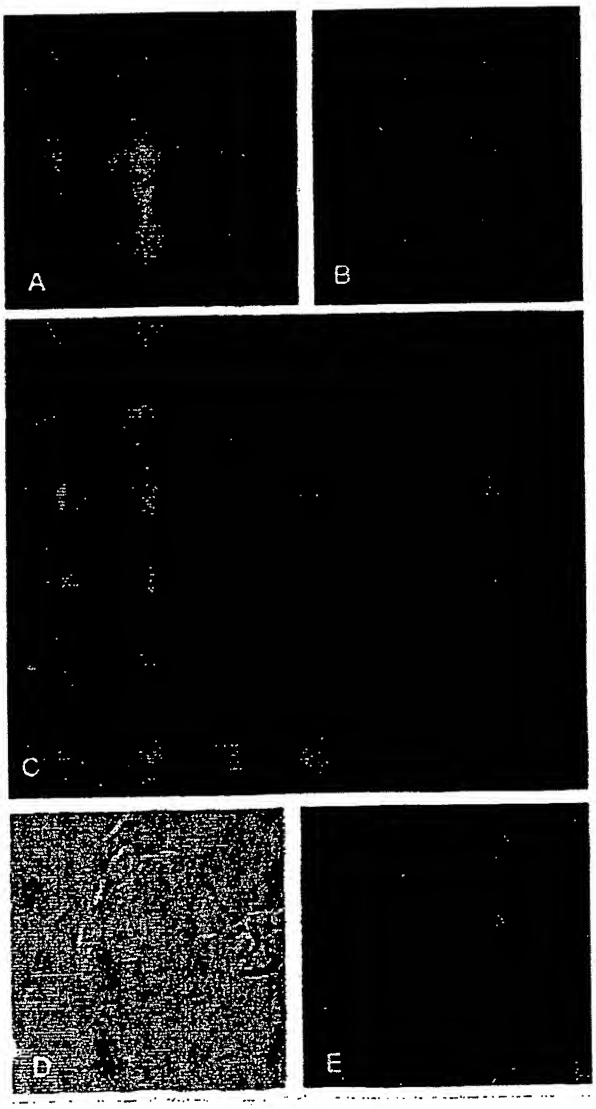

FIG. 10A

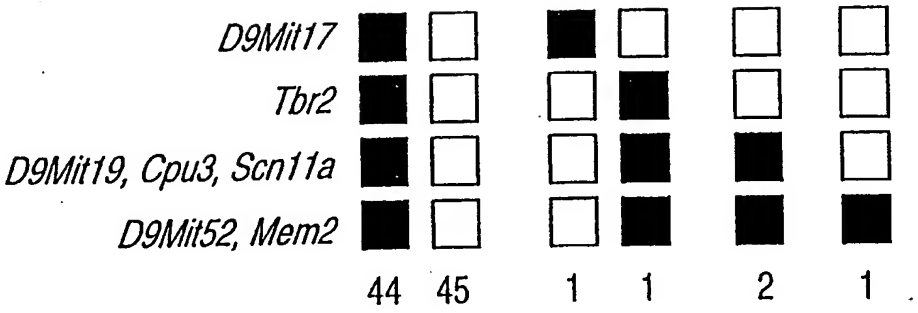

FIG. 10B

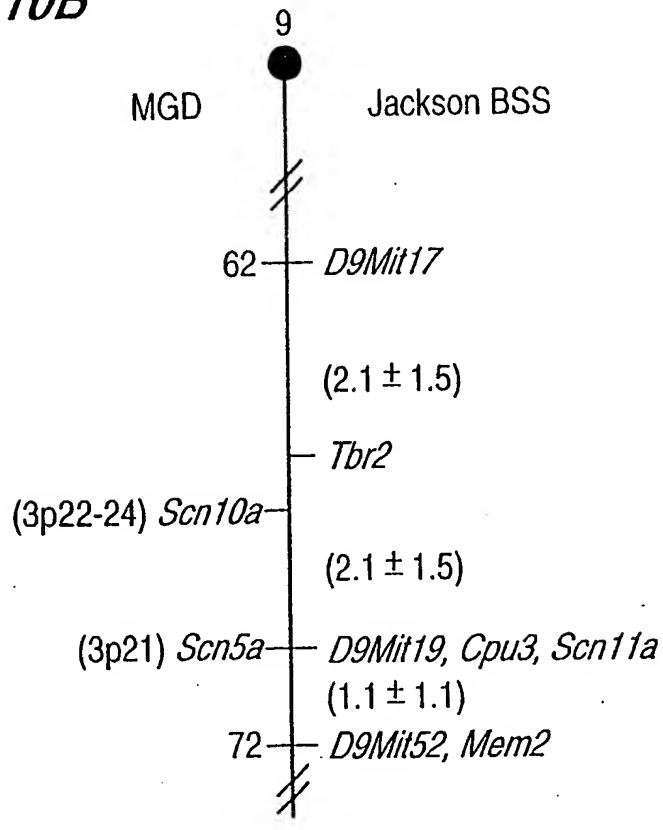

FIG. 11A

Sequence of human NaN cDNA. Open reading frame (cdc) Extends from position 31 (ATG) to the termination codon TGA at Position 5400.

```
   1  ATCTGCTCAA GCCAGGAATC TCGGGTGAAG ATGGATGACA GATGCTACCC
  51  AGTAATCTTT CCAGATGAGC GGAATTTCCG CCCCTTCACT TCCGACTCTC
 101  TGGCTGCAAT TGAGAAGCGG ATTGCCATCC AAAAGGAGAA AAAGAAGTCT
 151  AAAGACCAGA CAGGAGAAGT ACCCCAGCCT CGGCCTCAGC TTGACCTAAA
 201  GGCCTCCAGG AAGTTGCCCA AGCTCTATGG CGACATTCCT CGTGAGCTCA
 251  TAGGAAAGCC TCTGGAAGAC TTGGACCCAT TCTACCGAAA TCATAAGACA
 301  TTTATGGTGT TAAACAGAAA GAGGACAATC TACCGCTTCA GTGCCAAGCA
 351  TGCCTTGTTC ATTTTTGGGC CTTTCAATTC AATCAGAAGT TTAGCCATTA
 401  GAGTCTCAGT CCATTCATTG TTCAGCATGT TCATTATCGG CACCGTTATC
 451  ATCAACTGCG TGTTCATGGC TACAGGGCCT GCTAAAAACA GCAACAGTAA
 501  CAATACTGAC ATTGCAGAGT GTGTCTTCAC TGGGATTTAT ATTTTTGAAG
 551  CTTTGATTAA AATATTGGCA AGAGGTTTCA TTCTGGATGA GTTTTCTTTC
 601  CTTCGAGATC CATGGAACTG GCTGGACTCC ATTGTCATTG GAATAGCGAT
 651  TGTGTCATAT ATTCCAGGAA TCACCATCAA ACTATTGCCC CTGCGTACCT
 701  TCCGTGTGTT CAGAGCTTTG AAAGCAATTT CAGTAGTTTC ACGTCTGAAG
 751  GTCATCGTGG GGGCCTTGCT ACGCTCTGTG AAGAAGCTGG TCAACGTGAT
 801  TATCCTCACC TTCTTTTGCC TCAGCATCTT TGCCCTGGTA GGTCAGCAGC
 851  TCTTCATGGG AAGTCTGAAC CTGAAATGCA TCTCGAGGGA CTGTAAAAAT
 901  ATCAGTAACC CGGAAGCTTA TGACCATTGC TTTGAAAAGA AAGAAAATTC
 951  ACCTGAATTC AAAATGTGTG GCATCTGGAT GGGTAACAGT GCCTGTTCCA
1001  TACAATATGA ATGTAAGCAC ACCAAAATTA ATCCTGACTA TAATTATACG
1051  AATTTTGACA ACTTTGGCTG GTCTTTTCTT GCCATGTTCC GGCTGATGAC
1101  CCAAGATTCC TGGGAGAAGC TTTATCAACA GACCCTGCGT ACTACTGGGC
1151  TCTACTCAGT CTTCTTCTTC ATTGTGGTCA TTTTCCTGGG CTCCTTCTAC
1201  CTGATTAACT TAACCCTGGC TGTTGTTACC ATGGCATATG AGGAGCAGAA
```

FIG. IIA-2 23/28

1251 CAAGAATGTA GCTGCAGAGA TAGAGGCCAA GGAAAAGATG TTTCAGGAAG

1301 CCCAGCAGCT GTTAAAGGAG GAAAAGGAGG CTCTGGTTGC CATGGGAATT

1351 GACAGAAGTT CACTTACTTC CCTTGAAACA TCATATTTTA CCCCAAAAAA

1401 GAGAAAGCTC TTTGGTAATA AGAAAAGGAA GTCCTTCTTT TTGAGAGAGT

1451 CTGGGAAAGA CCAGCCTCCT GGGTCAGATT CTGATGAAGA TTGCCAAAAA

1501 AAGCCACAGC TCCTAGAGCA AACCAAACGA CTGTCCCAGA ATCTATCACT

1551 GGACCACTTT GATGAGCATG GAGATCCTCT CCAAAGGCAG AGAGCACTGA

1601 GTGCTGTCAG CATCCTCACC ATCACCATGA AGGAACAAGA AAAATCACAA

1651 GAGCCTTGTC TCCCTTGTGG AGAAAACCTG GCATCCAAGT ACCTCGTGTG

1701 GAACTGTTGC CCCCAGTGGC TGTGCGTTAA GAAGGTCCTG AGAACTGTGA

1751 TGACTGACCC GTTTACTGAG CTGGCCATCA CCATCTGCAT CATCATCAAC

1801 ACTGTCTTCT TGGCCATGGA GCATGACAAG ATGGAGGCCA GTTTTGAGAA

1851 GATGTTGAAT ATAGGGAATT TGGTTTTCAC TAGCATTTTT ATAGCAGAAA

1901 TGTGCCTAAA AATCATTGCG CTCGATCCCT ACCACTACTT TCGCCGAGGC

1951 TGGAACATTT TTGACAGCAT TGTTGCTCTT CTGAGTTTTG CAGATGTAAT

2001 GAACTGTGTA CTTCAAAAGA GAAGCTGGCC ATTCTTGCGT TCCTTCAGAG

2051 TGCTCAGGGT CTTCAAGTTA GCCAAATCCT GGCCAACTTT GAACACACTA

2101 ATTAAGATAA TCGGCAACTC TGTCGGAGCC CTTGGAAGCC TGACTGTGGT

2151 CCTGGTCATT GTGATCTTTA TTTTCTCAGT AGTTGGCATG CAGCTTTTTG

2201 GCCGTAGCTT CAATTCCCAA AAGAGTCCAA AACTCTGTAA CCCGACAGGC

2251 CCGACAGTCT CATGTTTACG GCACTGGCAC ATGGGGGATT TCTGGCACTC

2301 CTTCCTAGTG GTATTCCGCA TCCTCTGCGG GGAATGGATC GAAAATATGT

2351 GGGAATGTAT GCAAGAAGCG AATGCATCAT CATCATTGTG TGTTATTGTC

2401 TTCATATTGA TCACGGTGAT AGGAAAACTT GTGGTGCTCA ACCTCTTCAT

2451 TGCCTTACTG CTCAATTCCT TTAGCAATGA GGAAAGAAAT GGAAACTTAG

2501 AAGGAGAGGC CAGGAAAACT AAAGTCCAGT TAGCACTGGA TCGATTCCGC

2551 CGGGCTTTTT GTTTTGTGAG ACACACTCTT GAGCATTTCT GTCACAAGTG

FIG. IIA-3

```
2601  GTGCAGGAAG CAAAACTTAC CACAGCAAAA AGAGGTGGCA GGAGGCTGTG
2651  CTGCACAAAG CAAAGACATC ATTCCCCTGG TCATGGAGAT GAAAAGGGGC
2701  TCAGAGACCC AGGAGGAGCT TGGTATACTA ACCTCTGTAC CAAAGACCCT
2751  GGGCGTCAGG CATGATTGGA CTTGGTTGGC ACCACTTGCG GAGGAGGAAG
2801  ATGACGTTGA ATTTTCTGGT GAAGATAATG CACAGCGCAT CACACAACCT
2851  GAGCCTGAAC AACAGGCCTA TGAGCTCCAT CAGGAGAACA AGAAGCCCAC
2901  GAGCCAGAGA GTTCAAAGTG TGGAAATTGA CATGTTCTCT GAAGATGAGC
2951  CTCATCTGAC CATACAGGAT CCCCGAAAGA AGTCTGATGT TACCAGTATA
3001  CTATCAGAAT GTAGCACCAT TGATCTTCAG GATGGCTTTG GATGGTTACC
3051  TGAGATGGTT CCCAAAAAGC AACCAGAGAG ATGTTTGCCC AAAGGCTTTG
3101  GTTGCTGCTT TCCATGCTGT AGCGTGGACA AGAGAAAGCC TCCCTGGGTC
3151  ATTTGGTGGA ACCTGCGGAA AACCTGCTAC CAAATAGTGA AACACAGCTG
3201  GTTTGAGAGC TTTATTATCT TTGTGATTCT GCTGAGCAGT GGGGCACTGA
3251  TATTTGAAGA TGTTCACCTT GAGAACCAAC CCAAAATCCA AGAATTACTA
3301  AATTGTACTG ACATTATTTT TACACATATT TTTATCCTGG AGATGGTACT
3351  AAAATGGGTA GCCTTCGGAT TTGGAAAGTA TTTCACCAGT GCCTGGTGCT
3401  GCCTTGATTT CATCATTGTG ATTGTCTCTG TGACCACCCT CATTAACTTA
3451  ATGGAATTGA AGTCCTTCCG GACTCTACGA GCACTGAGGC CTCTTCGTGC
3501  GCTGTCCCAG TTTGAAGGAA TGAAGGTGGT GGTCAATGCT CTCATAGGTG
3551  CCATACCTGC CATTCTGAAT GTTTTGCTTG TCTGCCTCAT TTTCTGGCTC
3601  GTATTTTGTA TTCTGGGAGT ATACTTCTTT TCTGGAAAAT TTGGGAAATG
3651  CATTAATGGA ACAGACTCAG TTATAAATTA TACCATCATT ACAAATAAAA
3701  GTCAATGTGA AAGTGGCAAT TTCTCTTGGA TCAACCAGAA AGTCAACTTT
3751  GACAATGTGG GAAATGCTTA CCTCGCTCTG CTGCAAGTGG CAACATTTAA
3801  GGGCTGGATG GATATTATAT ATGCAGCTGT TGATTCCACA GAGAAAGAAC
3851  AACAGCCAGA GTTTGAGAGC AATTCACTCG GTTACATTTA CTTCGTAGTC
3901  TTTATCATCT TTGGCTCATT CTTCACTCTG AATCTCTTCA TTGGCGTTAT
3951  CATTGACAAC TTCAACCAAC AGCAGAAAAA GTTAGGTGGC CAAGACATTT
```

FIG. 11A-4

```
4001  TTATGACAGA AGAACAGAAG AAATACTATA ATGCAATGAA AAAATTAGGA
4051  TCCAAAAAAC CTCAAAAACC CATTCCACGG CCTCTGAACA AATGTCAAGG
4101  TCTCGTGTTC GACATAGTCA CAAGCCAGAT CTTTGACATC ATCATCATAA
4151  GTCTCATTAT CCTAAACATG ATTAGCATGA TGGCTGAATC ATACAACCAA
4201  CCCAAAGCCA TGAAATCCAT CCTTGACCAT CTCAACTGGG TCTTTGTGGT
4251  CATCTTTACG TTAGAATGTC TCATCAAAAT CTTTGCTTTG AGGCAATACT
4301  ACTTCACCAA TGGCTGGAAT TTATTTGACT GTGTGGTCGT GCTTCTTTCC
4351  ATTGTTAGTA CAATGATTTC TACCTTGGAA AATCAGGAGC ACATTCCTTT
4401  CCCTCCGACG CTCTTCAGAA TTGTCCGCTT GGCTCGGATT GGCCGAATCC
4451  TGAGGCTTGT CCGGGCTGCA CGAGGAATCA GGACTCTCCT CTTTGCTCTG
4501  ATGATGTCGC TTCCTTCTCT GTTCAACATT GGTCTTCTAC TCTTTCTGAT
4551  TATGTTTATC TATGCCATTC TGGGTATGAA CTGGTTTTCC AAAGTGAATC
4601  CAGAGTCTGG AATCGATGAC ATATTCAACT TCAAGACTTT TGCCAGCAGC
4651  ATGCTCTGTC TCTTCCAGAT AAGCACATCA GCAGGTTGGG ATTCCCTGCT
4701  CAGCCCCATG CTGCGATCAA AAGAATCATG TAACTCTTCC TCAGAAAACT
4751  GCCACCTCCC TGGCATAGCC ACATCCTACT TTGTCAGTTA CATTATCATC
4801  TCCTTTCTCA TTGTTGTCAA CATGTACATT GCTGTGATTT TAGAGAACTT
4851  CAATACAGCC ACTGAAGAAA GTGAGGACCC TTTGGGTGAA GATGACTTTG
4901  ACATATTTTA TGAAGTGTGG GAAAAGTTTG ACCCAGAAGC AACACAATTT
4951  ATCAAATATT CTGCCCTTTC TGACTTTGCT GATGCCTTGC CTGAGCCTTT
5001  GCGTGTCGCA AAGCCAAATA AATATCAATT TCTAGTAATG GACTTGCCCA
5051  TGGTGAGTGA AGATCGCCTC CACTGCATGG ATATTCTTTT CGCCTTCACC
5101  GCTAGGGTAC TCGGTGGCTC TGATGGCCTA GATAGTATGA AAGCAATGAT
5151  GGAAGAGAAG TTCATGGAAG CCAATCCTCT CAAGAAGTTG TATGAACCCA
5201  TAGTCACCAC CACCAAGAGA AAGGAAGAGG AAAGAGGTGC TGCTATTATT
5251  CAAAAGGCCT TTCGAAAGTA CATGATGAAG GTGACCAAGG GTGACCAAGG
5301  TGACCAAAAT GACTTGGAAA ACGGGCCTCA TTCACCACTC CAGACTCTTT
```

FIG. IIA-5

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5351  GCAATGGAGA CTTGTCTAGC TTTGGGGTGG CCAAGGGCAA GGTCCACTGT
5401  GACTGAGCCC TCACCTCCAC GCCTACCTCA TAGCTTCACA GCCTTGCCTT
5451  CAGCCTCTGA GCTCCAGGGG TCAGCAGCTT AGTGTATCAA CAGGGAGTGG
5501  ATTCACCAAA TTAGCCATTC CATTTTCTTT TCTGGCTAAA ATAAATGATA
5551  TTTCAATTTC ATTTTAAATG ATACTTACAG AGATATAAGA TAAGGCTACT
5601  TGACAACCAG TGGTACTATT ATAATAAGGA AGAAGACACC AGGAAGGACT
5651  GTAAAAGGAC ATACCAATTT TAGGATTGAA ATAGTTCAGG CCGGGCGCAG
5701  TGGCTCATGC CTGTAATCCC AGCACTTTGA GAGGCCAAGG CAGGTGGATC
5751  ACGAGGTCAA GAGATCGAGA CCATCCTGGC CAACATGATG AAACTCCGTC
5801  TCTCTAAAAA TACAAAAATT AGCTGGGCAT GGTGGCGTGC GCCTGTAGTC
5851  CCACTACTTG
```

FIG. IIB

Sequence of human NaN.

```
   1  MDDRCYPVIF PDERNFRPFT SDSLAAIEKR IAIQKEKKKS KDQTGEVPQP
  51  RPQLDLKASR KLPKLYGDIP RELIGKPLED LDPFYRNHKT FMVLNRKRTI
 101  YRFSAKHALF IFGPFNSIRS LAIRVSVHSL FSMFIIGTVI INCVFMATGP
 151  AKNSNSNNTD IAECVFTGIY IFEALIKILA RGFILDEFSF LRDPWNWLDS
 201  IVIGIAIVSY IPGITIKLLP LRTFRVFRAL KAISVVSRLK VIVGALLRSV
 251  KKLVNVIILT FFCLSIFALV GQQLFMGSLN LKCISRDCKN ISNPEAYDHC
 301  FEKKENSPEF KMCGIWMGNS ACSIQYECKH TKINPDYNYT NFDNFGWSFL
 351  AMFRLMTQDS WEKLYQQTLR TTGLYSVFFF IVVIFLGSFY LINLTLAVVT
 401  MAYEEQNKNV AAEIEAKEKM FQEAQQLLKE EKEALVAMGI DRSSLTSLET
 451  SYFTPKKRKL FGNKKRKSFF LRESGKDQPP GSDSDEDCQK KPQLLEQTKR
 501  LSQNLSLDHF DEHGDPLQRQ RALSAVSILT ITMKEQEKSQ EPCLPCGENL
 551  ASKYLVWNCC PQWLCVKKVL RTVMTDPFTE LAITICIIIN TVFLAMEHHK
 601  MEASFEKMLN IGNLVFTSIF IAEMCLKIIA LDPYHYFRRG WNIFDSIVAL
 651  LSFADVMNCV LQKRSWPFLR SFRVLRVFKL AKSWPTLNTL IKIIGNSVGA
 701  LGSLTVVLVI VIFIFSVVGM QLFGRSFNSQ KSPKLCNPTG PTVSCLRHWH
 751  MGDFWHSFLV VFRILCGEWI ENMWECMQEA NASSSLCVIV FILITVIGKL
 801  VVLNLFIALL LNSFSNEERN GNLEGEARKT KVQLALDRFR RAFCFVRHTL
 851  EHFCHKWCRK QNLPQQKEVA GGCAAQSKDI IPLVMEMKRG SETQEELGIL
 901  TSVPKTLGVR HDWTWLAPLA EEEDDVEFSG EDNAQRITQP EPEQQAYELH
 951  QENKKPTSQR VQSVEIDMFS EDEPHLTIQD PRKKSDVTSI LSECSTIDLQ
1001  DGFGWLPEMV PKKQPERCLP KGFGCCFPCC SVDKRKPPWV IWWNLRKTCY
1051  QIVKHSWFES FIIFVILLSS GALIFEDVHL ENQPKIQELL NCTDIIFTHI
1101  FILEMVLKWV AFGFGKYFTS AWCCLDFIIV IVSVTTLINL MELKSFRTLR
1151  ALRPLRALSQ FEGMKVVVNA LIGAIPAILN VLLVCLIFWL VFCILGVYFF
```

FIG. 11B-2

1201 SGKFGKCING TDSVINYTII TNKSQCESGN FSWINQKVNF DNVGNAYLAL

1251 LQVATFKGWM DIIYAAVDST EKEQQPEFES NSLGYIYFVV FIIFGSFFTL

1301 NLFIGVIIDN FNQQQKKLGG QDIFMTEEQK KYYNAMKKLG SKKPQKPIPR

1351 PLNKCQGLVF DIVTSQIFDI IIISLIILNM ISMMAESYNQ PKAMKSILDH

1401 LNWVFVVIFT LECLIKIFAL RQYYFTNGWN LFDCVVVLLS IVSTMISTLE

1451 NQEHIPFPPT LFRIVRLARI GRILRLVRAA RGIRTLLFAL MMSLPSLFNI

1501 GLLLFLIMFI YAILGMNWFS KVNPESGIDD IFNFKTFASS MLCLFQISTS

1551 AGWDSLLSPM LRSKESCNSS SENCHLPGIA TSYFVSYIII SFLIVVNMYI

1601 AVILENFNTA TEESEDPLGE DDFDIFYEVW EKFDPEATQF IKYSALSDFA

1651 DALPEPLRVA KPNKYQFLVM DLPMVSEDRL HCMDILFAFT ARVLGGSDGL

1701 DSMKAMMEEK FMEANPLKKL YEPIVTTTKR KEEERGAAII QKAFRKYMMK

1751 VTKGDQGDQN DLENGPHSPL QTLCNGDLSS FGVAKGKVHC D.

<400> 38
Met Trp Xaa Cys Met Glu Val
1 5

<210> 39
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: rat NaN forward primer

<400> 39
ccctgctgcg ctcggtgaag aa 22

<210> 40
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: a.a. seq. used to derive epitope for polyclonal antibody

<400> 40
Cys Gly Pro Asn Pro Ala Ser Asn Lys Asp Cys Phe Glu Lys Glu Lys
1 5 10 15

Asp Ser Glu Asp
20

<210> 41
<211> 5860
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (31)..(5403)
<223> full length cDNA sequence for human NaN

<400> 41
atctgctcaa gccaggaatc tcgggtgaag atg gat gac aga tgc tac cca gta 54
Met Asp Asp Arg Cys Tyr Pro Val
1 5

atc ttt cca gat gag cgg aat ttc cgc ccc ttc act tcc gac tct ctg 102
Ile Phe Pro Asp Glu Arg Asn Phe Arg Pro Phe Thr Ser Asp Ser Leu
10 15 20

gct gca att gag aag cgg att gcc atc caa aag gag aaa aag aag tct 150
Ala Ala Ile Glu Lys Arg Ile Ala Ile Gln Lys Glu Lys Lys Lys Ser

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25                  30                  35                  40

aaa gac cag aca gga gaa gta ccc cag cct cgg cct cag ctt gac cta   198
Lys Asp Gln Thr Gly Glu Val Pro Gln Pro Arg Pro Gln Leu Asp Leu
                45                  50                  55

aag gcc tcc agg aag ttg ccc aag ctc tat ggc gac att cct cgt gag   246
Lys Ala Ser Arg Lys Leu Pro Lys Leu Tyr Gly Asp Ile Pro Arg Glu
            60                  65                  70

ctc ata gga aag cct ctg gaa gac ttg gac cca ttc tac cga aat cat   294
Leu Ile Gly Lys Pro Leu Glu Asp Leu Asp Pro Phe Tyr Arg Asn His
        75                  80                  85

aag aca ttt atg gtg tta aac aga aag agg aca atc tac cgc ttc agt   342
Lys Thr Phe Met Val Leu Asn Arg Lys Arg Thr Ile Tyr Arg Phe Ser
    90                  95                  100

gcc aag cat gcc ttg ttc att ttt ggg cct ttc aat tca atc aga agt   390
Ala Lys His Ala Leu Phe Ile Phe Gly Pro Phe Asn Ser Ile Arg Ser
105                 110                 115                 120

tta gcc att aga gtc tca gtc cat tca ttg ttc agc atg ttc att atc   438
Leu Ala Ile Arg Val Ser Val His Ser Leu Phe Ser Met Phe Ile Ile
                125                 130                 135

ggc acc gtt atc atc aac tgc gtg ttc atg gct aca ggg cct gct aaa   486
Gly Thr Val Ile Ile Asn Cys Val Phe Met Ala Thr Gly Pro Ala Lys
            140                 145                 150

aac agc aac agt aac aat act gac att gca gag tgt gtc ttc act ggg   534
Asn Ser Asn Ser Asn Asn Thr Asp Ile Ala Glu Cys Val Phe Thr Gly
        155                 160                 165

att tat att ttt gaa gct ttg att aaa ata ttg gca aga ggt ttc att   582
Ile Tyr Ile Phe Glu Ala Leu Ile Lys Ile Leu Ala Arg Gly Phe Ile
    170                 175                 180

ctg gat gag ttt tct ttc ctt cga gat cca tgg aac tgg ctg gac tcc   630
Leu Asp Glu Phe Ser Phe Leu Arg Asp Pro Trp Asn Trp Leu Asp Ser
185                 190                 195                 200

att gtc att gga ata gcg att gtg tca tat att cca gga atc acc atc   678
Ile Val Ile Gly Ile Ala Ile Val Ser Tyr Ile Pro Gly Ile Thr Ile
                205                 210                 215

aaa cta ttg ccc ctg cgt acc ttc cgt gtg ttc aga gct ttg aaa gca   726
Lys Leu Leu Pro Leu Arg Thr Phe Arg Val Phe Arg Ala Leu Lys Ala
            220                 225                 230

att tca gta gtt tca cgt ctg aag gtc atc gtg ggg gcc ttg cta cgc   774
Ile Ser Val Val Ser Arg Leu Lys Val Ile Val Gly Ala Leu Leu Arg
        235                 240                 245

tct gtg aag aag ctg gtc aac gtg att atc ctc acc ttc ttt tgc ctc   822
Ser Val Lys Lys Leu Val Asn Val Ile Ile Leu Thr Phe Phe Cys Leu
```

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    250                 255                 260

agc atc ttt gcc ctg gta ggt cag cag ctc ttc atg gga agt ctg aac   870
Ser Ile Phe Ala Leu Val Gly Gln Gln Leu Phe Met Gly Ser Leu Asn
265                 270                 275                 280

ctg aaa tgc atc tcg agg gac tgt aaa aat atc agt aac ccg gaa gct   918
Leu Lys Cys Ile Ser Arg Asp Cys Lys Asn Ile Ser Asn Pro Glu Ala
                285                 290                 295

tat gac cat tgc ttt gaa aag aaa gaa aat tca cct gaa ttc aaa atg   966
Tyr Asp His Cys Phe Glu Lys Lys Glu Asn Ser Pro Glu Phe Lys Met
            300                 305                 310

tgt ggc atc tgg atg ggt aac agt gcc tgt tcc ata caa tat gaa tgt   1014
Cys Gly Ile Trp Met Gly Asn Ser Ala Cys Ser Ile Gln Tyr Glu Cys
        315                 320                 325

aag cac acc aaa att aat cct gac tat aat tat acg aat ttt gac aac   1062
Lys His Thr Lys Ile Asn Pro Asp Tyr Asn Tyr Thr Asn Phe Asp Asn
    330                 335                 340

ttt ggc tgg tct ttt ctt gcc atg ttc cgg ctg atg acc caa gat tcc   1110
Phe Gly Trp Ser Phe Leu Ala Met Phe Arg Leu Met Thr Gln Asp Ser
345                 350                 355                 360

tgg gag aag ctt tat caa cag acc ctg cgt act act ggg ctc tac tca   1158
Trp Glu Lys Leu Tyr Gln Gln Thr Leu Arg Thr Thr Gly Leu Tyr Ser
                365                 370                 375

gtc ttc ttc ttc att gtg gtc att ttc ctg ggc tcc ttc tac ctg att   1206
Val Phe Phe Phe Ile Val Val Ile Phe Leu Gly Ser Phe Tyr Leu Ile
            380                 385                 390

aac tta acc ctg gct gtt gtt acc atg gca tat gag gag cag aac aag   1254
Asn Leu Thr Leu Ala Val Val Thr Met Ala Tyr Glu Glu Gln Asn Lys
        395                 400                 405

aat gta gct gca gag ata gag gcc aag gaa aag atg ttt cag gaa gcc   1302
Asn Val Ala Ala Glu Ile Glu Ala Lys Glu Lys Met Phe Gln Glu Ala
    410                 415                 420

cag cag ctg tta aag gag gaa aag gag gct ctg gtt gcc atg gga att   1350
Gln Gln Leu Leu Lys Glu Glu Lys Glu Ala Leu Val Ala Met Gly Ile
425                 430                 435                 440

gac aga agt tca ctt act tcc ctt gaa aca tca tat ttt acc cca aaa   1398
Asp Arg Ser Ser Leu Thr Ser Leu Glu Thr Ser Tyr Phe Thr Pro Lys
                445                 450                 455

aag aga aag ctc ttt ggt aat aag aaa agg aag tcc ttc ttt ttg aga   1446
Lys Arg Lys Leu Phe Gly Asn Lys Lys Arg Lys Ser Phe Phe Leu Arg
            460                 465                 470

gag tct ggg aaa gac cag cct cct ggg tca gat tct gat gaa gat tgc   1494
Glu Ser Gly Lys Asp Gln Pro Pro Gly Ser Asp Ser Asp Glu Asp Cys
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        475                 480                 485

caa aaa aag cca cag ctc cta gag caa acc aaa cga ctg tcc cag aat   1542
Gln Lys Lys Pro Gln Leu Leu Glu Gln Thr Lys Arg Leu Ser Gln Asn
    490                 495                 500

cta tca ctg gac cac ttt gat gag cat gga gat cct ctc caa agg cag   1590
Leu Ser Leu Asp His Phe Asp Glu His Gly Asp Pro Leu Gln Arg Gln
505                 510                 515                 520

aga gca ctg agt gct gtc agc atc ctc acc atc acc atg aag gaa caa   1638
Arg Ala Leu Ser Ala Val Ser Ile Leu Thr Ile Thr Met Lys Glu Gln
                525                 530                 535

gaa aaa tca caa gag cct tgt ctc cct tgt gga gaa aac ctg gca tcc   1686
Glu Lys Ser Gln Glu Pro Cys Leu Pro Cys Gly Glu Asn Leu Ala Ser
            540                 545                 550

aag tac ctc gtg tgg aac tgt tgc ccc cag tgg ctg tgc gtt aag aag   1734
Lys Tyr Leu Val Trp Asn Cys Cys Pro Gln Trp Leu Cys Val Lys Lys
        555                 560                 565

gtc ctg aga act gtg atg act gac ccg ttt act gag ctg gcc atc acc   1782
Val Leu Arg Thr Val Met Thr Asp Pro Phe Thr Glu Leu Ala Ile Thr
    570                 575                 580

atc tgc atc atc atc aac act gtc ttc ttg gcc atg gag cat cac aag   1830
Ile Cys Ile Ile Ile Asn Thr Val Phe Leu Ala Met Glu His His Lys
585                 590                 595                 600

atg gag gcc agt ttt gag aag atg ttg aat ata ggg aat ttg gtt ttc   1878
Met Glu Ala Ser Phe Glu Lys Met Leu Asn Ile Gly Asn Leu Val Phe
                605                 610                 615

act agc att ttt ata gca gaa atg tgc cta aaa atc att gcg ctc gat   1926
Thr Ser Ile Phe Ile Ala Glu Met Cys Leu Lys Ile Ile Ala Leu Asp
            620                 625                 630

ccc tac cac tac ttt cgc cga ggc tgg aac att ttt gac agc att gtt   1974
Pro Tyr His Tyr Phe Arg Arg Gly Trp Asn Ile Phe Asp Ser Ile Val
        635                 640                 645

gct ctt ctg agt ttt gca gat gta atg aac tgt gta ctt caa aag aga   2022
Ala Leu Leu Ser Phe Ala Asp Val Met Asn Cys Val Leu Gln Lys Arg
    650                 655                 660

agc tgg cca ttc ttg cgt tcc ttc aga gtg ctc agg gtc ttc aag tta   2070
Ser Trp Pro Phe Leu Arg Ser Phe Arg Val Leu Arg Val Phe Lys Leu
665                 670                 675                 680

gcc aaa tcc tgg cca act ttg aac aca cta att aag ata atc ggc aac   2118
Ala Lys Ser Trp Pro Thr Leu Asn Thr Leu Ile Lys Ile Ile Gly Asn
                685                 690                 695

tct gtc gga gcc ctt gga agc ctg act gtg gtc ctg gtc att gtg atc   2166
Ser Val Gly Ala Leu Gly Ser Leu Thr Val Val Leu Val Ile Val Ile
```

```
            700                 705                 710

ttt att ttc tca gta gtt ggc atg cag ctt ttt ggc cgt agc ttc aat   2214
Phe Ile Phe Ser Val Val Gly Met Gln Leu Phe Gly Arg Ser Phe Asn
        715                 720                 725

tcc caa aag agt cca aaa ctc tgt aac ccg aca ggc ccg aca gtc tca   2262
Ser Gln Lys Ser Pro Lys Leu Cys Asn Pro Thr Gly Pro Thr Val Ser
    730                 735                 740

tgt tta cgg cac tgg cac atg ggg gat ttc tgg cac tcc ttc cta gtg   2310
Cys Leu Arg His Trp His Met Gly Asp Phe Trp His Ser Phe Leu Val
745                 750                 755                 760

gta ttc cgc atc ctc tgc ggg gaa tgg atc gaa aat atg tgg gaa tgt   2358
Val Phe Arg Ile Leu Cys Gly Glu Trp Ile Glu Asn Met Trp Glu Cys
                765                 770                 775

atg caa gaa gcg aat gca tca tca tca ttg tgt gtt att gtc ttc ata   2406
Met Gln Glu Ala Asn Ala Ser Ser Ser Leu Cys Val Ile Val Phe Ile
            780                 785                 790

ttg atc acg gtg ata gga aaa ctt gtg gtg ctc aac ctc ttc att gcc   2454
Leu Ile Thr Val Ile Gly Lys Leu Val Val Leu Asn Leu Phe Ile Ala
        795                 800                 805

tta ctg ctc aat tcc ttt agc aat gag gaa aga aat gga aac tta gaa   2502
Leu Leu Leu Asn Ser Phe Ser Asn Glu Glu Arg Asn Gly Asn Leu Glu
    810                 815                 820

gga gag gcc agg aaa act aaa gtc cag tta gca ctg gat cga ttc cgc   2550
Gly Glu Ala Arg Lys Thr Lys Val Gln Leu Ala Leu Asp Arg Phe Arg
825                 830                 835                 840

cgg gct ttt tgt ttt gtg aga cac act ctt gag cat ttc tgt cac aag   2598
Arg Ala Phe Cys Phe Val Arg His Thr Leu Glu His Phe Cys His Lys
                845                 850                 855

tgg tgc agg aag caa aac tta cca cag caa aaa gag gtg gca gga ggc   2646
Trp Cys Arg Lys Gln Asn Leu Pro Gln Gln Lys Glu Val Ala Gly Gly
            860                 865                 870

tgt gct gca caa agc aaa gac atc att ccc ctg gtc atg gag atg aaa   2694
Cys Ala Ala Gln Ser Lys Asp Ile Ile Pro Leu Val Met Glu Met Lys
        875                 880                 885

agg ggc tca gag acc cag gag gag ctt ggt ata cta acc tct gta cca   2742
Arg Gly Ser Glu Thr Gln Glu Glu Leu Gly Ile Leu Thr Ser Val Pro
    890                 895                 900

aag acc ctg ggc gtc agg cat gat tgg act tgg ttg gca cca ctt gcg   2790
Lys Thr Leu Gly Val Arg His Asp Trp Thr Trp Leu Ala Pro Leu Ala
905                 910                 915                 920

gag gag gaa gat gac gtt gaa ttt tct ggt gaa gat aat gca cag cgc   2838
Glu Glu Glu Asp Asp Val Glu Phe Ser Gly Glu Asp Asn Ala Gln Arg
```

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                925                 930                 935

atc aca caa cct gag cct gaa caa cag gcc tat gag ctc cat cag gag   2886
Ile Thr Gln Pro Glu Pro Glu Gln Gln Ala Tyr Glu Leu His Gln Glu
            940                 945                 950

aac aag aag ccc acg agc cag aga gtt caa agt gtg gaa att gac atg   2934
Asn Lys Lys Pro Thr Ser Gln Arg Val Gln Ser Val Glu Ile Asp Met
        955                 960                 965

ttc tct gaa gat gag cct cat ctg acc ata cag gat ccc cga aag aag   2982
Phe Ser Glu Asp Glu Pro His Leu Thr Ile Gln Asp Pro Arg Lys Lys
    970                 975                 980

tct gat gtt acc agt ata cta tca gaa tgt agc acc att gat ctt cag   3030
Ser Asp Val Thr Ser Ile Leu Ser Glu Cys Ser Thr Ile Asp Leu Gln
985                 990                 995                1000

gat ggc ttt gga tgg tta cct gag atg gtt ccc aaa aag caa cca gag   3078
Asp Gly Phe Gly Trp Leu Pro Glu Met Val Pro Lys Lys Gln Pro Glu
                1005                1010                1015

aga tgt ttg ccc aaa ggc ttt ggt tgc tgc ttt cca tgc tgt agc gtg   3126
Arg Cys Leu Pro Lys Gly Phe Gly Cys Cys Phe Pro Cys Cys Ser Val
            1020                1025                1030

gac aag aga aag cct ccc tgg gtc att tgg tgg aac ctg cgg aaa acc   3174
Asp Lys Arg Lys Pro Pro Trp Val Ile Trp Trp Asn Leu Arg Lys Thr
        1035                1040                1045

tgc tac caa ata gtg aaa cac agc tgg ttt gag agc ttt att atc ttt   3222
Cys Tyr Gln Ile Val Lys His Ser Trp Phe Glu Ser Phe Ile Ile Phe
    1050                1055                1060

gtg att ctg ctg agc agt ggg gca ctg ata ttt gaa gat gtt cac ctt   3270
Val Ile Leu Leu Ser Ser Gly Ala Leu Ile Phe Glu Asp Val His Leu
1065                1070                1075                1080

gag aac caa ccc aaa atc caa gaa tta cta aat tgt act gac att att   3318
Glu Asn Gln Pro Lys Ile Gln Glu Leu Leu Asn Cys Thr Asp Ile Ile
                1085                1090                1095

ttt aca cat att ttt atc ctg gag atg gta cta aaa tgg gta gcc ttc   3366
Phe Thr His Ile Phe Ile Leu Glu Met Val Leu Lys Trp Val Ala Phe
            1100                1105                1110

gga ttt gga aag tat ttc acc agt gcc tgg tgc tgc ctt gat ttc atc   3414
Gly Phe Gly Lys Tyr Phe Thr Ser Ala Trp Cys Cys Leu Asp Phe Ile
        1115                1120                1125

att gtg att gtc tct gtg acc acc ctc att aac tta atg gaa ttg aag   3462
Ile Val Ile Val Ser Val Thr Thr Leu Ile Asn Leu Met Glu Leu Lys
    1130                1135                1140

tcc ttc cgg act cta cga gca ctg agg cct ctt cgt gcg ctg tcc cag   3510
Ser Phe Arg Thr Leu Arg Ala Leu Arg Pro Leu Arg Ala Leu Ser Gln
```

```
1145                1150                1155                1160

ttt gaa gga atg aag gtg gtg gtc aat gct ctc ata ggt gcc ata cct   3558
Phe Glu Gly Met Lys Val Val Val Asn Ala Leu Ile Gly Ala Ile Pro
                1165                1170                1175

gcc att ctg aat gtt ttg ctt gtc tgc ctc att ttc tgg ctc gta ttt   3606
Ala Ile Leu Asn Val Leu Leu Val Cys Leu Ile Phe Trp Leu Val Phe
            1180                1185                1190

tgt att ctg gga gta tac ttc ttt tct gga aaa ttt ggg aaa tgc att   3654
Cys Ile Leu Gly Val Tyr Phe Phe Ser Gly Lys Phe Gly Lys Cys Ile
        1195                1200                1205

aat gga aca gac tca gtt ata aat tat acc atc att aca aat aaa agt   3702
Asn Gly Thr Asp Ser Val Ile Asn Tyr Thr Ile Ile Thr Asn Lys Ser
    1210                1215                1220

caa tgt gaa agt ggc aat ttc tct tgg atc aac cag aaa gtc aac ttt   3750
Gln Cys Glu Ser Gly Asn Phe Ser Trp Ile Asn Gln Lys Val Asn Phe
1225                1230                1235                1240

gac aat gtg gga aat gct tac ctc gct ctg ctg caa gtg gca aca ttt   3798
Asp Asn Val Gly Asn Ala Tyr Leu Ala Leu Leu Gln Val Ala Thr Phe
                1245                1250                1255

aag ggc tgg atg gat att ata tat gca gct gtt gat tcc aca gag aaa   3846
Lys Gly Trp Met Asp Ile Ile Tyr Ala Ala Val Asp Ser Thr Glu Lys
            1260                1265                1270

gaa caa cag cca gag ttt gag agc aat tca ctc ggt tac att tac ttc   3894
Glu Gln Gln Pro Glu Phe Glu Ser Asn Ser Leu Gly Tyr Ile Tyr Phe
        1275                1280                1285

gta gtc ttt atc atc ttt ggc tca ttc ttc act ctg aat ctc ttc att   3942
Val Val Phe Ile Ile Phe Gly Ser Phe Phe Thr Leu Asn Leu Phe Ile
    1290                1295                1300

ggc gtt atc att gac aac ttc aac caa cag cag aaa aag tta ggt ggc   3990
Gly Val Ile Ile Asp Asn Phe Asn Gln Gln Gln Lys Lys Leu Gly Gly
1305                1310                1315                1320

caa gac att ttt atg aca gaa gaa cag aag aaa tac tat aat gca atg   4038
Gln Asp Ile Phe Met Thr Glu Glu Gln Lys Lys Tyr Tyr Asn Ala Met
                1325                1330                1335

aaa aaa tta gga tcc aaa aaa cct caa aaa ccc att cca cgg cct ctg   4086
Lys Lys Leu Gly Ser Lys Lys Pro Gln Lys Pro Ile Pro Arg Pro Leu
            1340                1345                1350

aac aaa tgt caa ggt ctc gtg ttc gac ata gtc aca agc cag atc ttt   4134
Asn Lys Cys Gln Gly Leu Val Phe Asp Ile Val Thr Ser Gln Ile Phe
        1355                1360                1365

gac atc atc atc ata agt ctc att atc cta aac atg att agc atg atg   4182
Asp Ile Ile Ile Ile Ser Leu Ile Ile Leu Asn Met Ile Ser Met Met
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   1370                1375                1380

gct gaa tca tac aac caa ccc aaa gcc atg aaa tcc atc ctt gac cat   4230
Ala Glu Ser Tyr Asn Gln Pro Lys Ala Met Lys Ser Ile Leu Asp His
1385                1390                1395                1400

ctc aac tgg gtc ttt gtg gtc atc ttt acg tta gaa tgt ctc atc aaa   4278
Leu Asn Trp Val Phe Val Val Ile Phe Thr Leu Glu Cys Leu Ile Lys
                1405                1410                1415

atc ttt gct ttg agg caa tac tac ttc acc aat ggc tgg aat tta ttt   4326
Ile Phe Ala Leu Arg Gln Tyr Tyr Phe Thr Asn Gly Trp Asn Leu Phe
            1420                1425                1430

gac tgt gtg gtc gtg ctt ctt tcc att gtt agt aca atg att tct acc   4374
Asp Cys Val Val Val Leu Leu Ser Ile Val Ser Thr Met Ile Ser Thr
        1435                1440                1445

ttg gaa aat cag gag cac att cct ttc cct ccg acg ctc ttc aga att   4422
Leu Glu Asn Gln Glu His Ile Pro Phe Pro Pro Thr Leu Phe Arg Ile
    1450                1455                1460

gtc cgc ttg gct cgg att ggc cga atc ctg agg ctt gtc cgg gct gca   4470
Val Arg Leu Ala Arg Ile Gly Arg Ile Leu Arg Leu Val Arg Ala Ala
1465                1470                1475                1480

cga gga atc agg act ctc ctc ttt gct ctg atg atg tcg ctt cct tct   4518
Arg Gly Ile Arg Thr Leu Leu Phe Ala Leu Met Met Ser Leu Pro Ser
                1485                1490                1495

ctg ttc aac att ggt ctt cta ctc ttt ctg att atg ttt atc tat gcc   4566
Leu Phe Asn Ile Gly Leu Leu Leu Phe Leu Ile Met Phe Ile Tyr Ala
            1500                1505                1510

att ctg ggt atg aac tgg ttt tcc aaa gtg aat cca gag tct gga atc   4614
Ile Leu Gly Met Asn Trp Phe Ser Lys Val Asn Pro Glu Ser Gly Ile
        1515                1520                1525

gat gac ata ttc aac ttc aag act ttt gcc agc agc atg ctc tgt ctc   4662
Asp Asp Ile Phe Asn Phe Lys Thr Phe Ala Ser Ser Met Leu Cys Leu
    1530                1535                1540

ttc cag ata agc aca tca gca ggt tgg gat tcc ctg ctc agc ccc atg   4710
Phe Gln Ile Ser Thr Ser Ala Gly Trp Asp Ser Leu Leu Ser Pro Met
1545                1550                1555                1560

ctg cga tca aaa gaa tca tgt aac tct tcc tca gaa aac tgc cac ctc   4758
Leu Arg Ser Lys Glu Ser Cys Asn Ser Ser Ser Glu Asn Cys His Leu
                1565                1570                1575

cct ggc ata gcc aca tcc tac ttt gtc agt tac att atc atc tcc ttt   4806
Pro Gly Ile Ala Thr Ser Tyr Phe Val Ser Tyr Ile Ile Ile Ser Phe
            1580                1585                1590

ctc att gtt gtc aac atg tac att gct gtg att tta gag aac ttc aat   4854
Leu Ile Val Val Asn Met Tyr Ile Ala Val Ile Leu Glu Asn Phe Asn
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      1595                1600                1605

aca gcc act gaa gaa agt gag gac cct ttg ggt gaa gat gac ttt gac   4902
Thr Ala Thr Glu Glu Ser Glu Asp Pro Leu Gly Glu Asp Asp Phe Asp
   1610                1615                1620

ata ttt tat gaa gtg tgg gaa aag ttt gac cca gaa gca aca caa ttt   4950
Ile Phe Tyr Glu Val Trp Glu Lys Phe Asp Pro Glu Ala Thr Gln Phe
1625                1630                1635                1640

atc aaa tat tct gcc ctt tct gac ttt gct gat gcc ttg cct gag cct   4998
Ile Lys Tyr Ser Ala Leu Ser Asp Phe Ala Asp Ala Leu Pro Glu Pro
               1645                1650                1655

ttg cgt gtc gca aag cca aat aaa tat caa ttt cta gta atg gac ttg   5046
Leu Arg Val Ala Lys Pro Asn Lys Tyr Gln Phe Leu Val Met Asp Leu
          1660                1665                1670

ccc atg gtg agt gaa gat cgc ctc cac tgc atg gat att ctt ttc gcc   5094
Pro Met Val Ser Glu Asp Arg Leu His Cys Met Asp Ile Leu Phe Ala
       1675                1680                1685

ttc acc gct agg gta ctc ggt ggc tct gat ggc cta gat agt atg aaa   5142
Phe Thr Ala Arg Val Leu Gly Gly Ser Asp Gly Leu Asp Ser Met Lys
   1690                1695                1700

gca atg atg gaa gag aag ttc atg gaa gcc aat cct ctc aag aag ttg   5190
Ala Met Met Glu Glu Lys Phe Met Glu Ala Asn Pro Leu Lys Lys Leu
1705                1710                1715                1720

tat gaa ccc ata gtc acc acc acc aag aga aag gaa gag gaa aga ggt   5238
Tyr Glu Pro Ile Val Thr Thr Thr Lys Arg Lys Glu Glu Glu Arg Gly
               1725                1730                1735

gct gct att att caa aag gcc ttt cga aag tac atg atg aag gtg acc   5286
Ala Ala Ile Ile Gln Lys Ala Phe Arg Lys Tyr Met Met Lys Val Thr
          1740                1745                1750

aag ggt gac caa ggt gac caa aat gac ttg gaa aac ggg cct cat tca   5334
Lys Gly Asp Gln Gly Asp Gln Asn Asp Leu Glu Asn Gly Pro His Ser
       1755                1760                1765

cca ctc cag act ctt tgc aat gga gac ttg tct agc ttt ggg gtg gcc   5382
Pro Leu Gln Thr Leu Cys Asn Gly Asp Leu Ser Ser Phe Gly Val Ala
   1770                1775                1780

aag ggc aag gtc cac tgt gac tgagccctca cctccacgcc tacctcatag      5433
Lys Gly Lys Val His Cys Asp
1785                1790

cttcacagcc ttgccttcag cctctgagct ccaggggtca gcagcttagt gtatcaacag 5493

ggagtggatt caccaaatta gccattccat tttcttttct ggctaaaata aatgatattt 5553

caatttcatt ttaaatgata cttacagaga tataagataa ggctacttga caaccagtgg 5613
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gccaaggcag gtggatcacg aggtcaagag atcgagacca tcctggccaa catgatgaaa 5793

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ctacttg 5860

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Ile Gln Lys Glu Lys Lys Lys Ser Lys Asp Gln Thr Gly Glu Val Pro
         35                  40                  45

Gln Pro Arg Pro Gln Leu Asp Leu Lys Ala Ser Arg Lys Leu Pro Lys
     50                  55                  60

Leu Tyr Gly Asp Ile Pro Arg Glu Leu Ile Gly Lys Pro Leu Glu Asp
 65                  70                  75                  80

Leu Asp Pro Phe Tyr Arg Asn His Lys Thr Phe Met Val Leu Asn Arg
                 85                  90                  95

Lys Arg Thr Ile Tyr Arg Phe Ser Ala Lys His Ala Leu Phe Ile Phe
            100                 105                 110

Gly Pro Phe Asn Ser Ile Arg Ser Leu Ala Ile Arg Val Ser Val His
        115                 120                 125

Ser Leu Phe Ser Met Phe Ile Ile Gly Thr Val Ile Ile Asn Cys Val
    130                 135                 140

Phe Met Ala Thr Gly Pro Ala Lys Asn Ser Asn Ser Asn Asn Thr Asp
145                 150                 155                 160

Ile Ala Glu Cys Val Phe Thr Gly Ile Tyr Ile Phe Glu Ala Leu Ile
                165                 170                 175

Lys Ile Leu Ala Arg Gly Phe Ile Leu Asp Glu Phe Ser Phe Leu Arg
            180                 185                 190

Asp Pro Trp Asn Trp Leu Asp Ser Ile Val Ile Gly Ile Ala Ile Val
        195                 200                 205
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Ser Tyr Ile Pro Gly Ile Thr Ile Lys Leu Leu Pro Leu Arg Thr Phe
    210                 215                 220

Arg Val Phe Arg Ala Leu Lys Ala Ile Ser Val Val Ser Arg Leu Lys
225                 230                 235                 240

Val Ile Val Gly Ala Leu Leu Arg Ser Val Lys Lys Leu Val Asn Val
                245                 250                 255

Ile Ile Leu Thr Phe Phe Cys Leu Ser Ile Phe Ala Leu Val Gly Gln
            260                 265                 270

Gln Leu Phe Met Gly Ser Leu Asn Leu Lys Cys Ile Ser Arg Asp Cys
        275                 280                 285

Lys Asn Ile Ser Asn Pro Glu Ala Tyr Asp His Cys Phe Glu Lys Lys
    290                 295                 300

Glu Asn Ser Pro Glu Phe Lys Met Cys Gly Ile Trp Met Gly Asn Ser
305                 310                 315                 320

Ala Cys Ser Ile Gln Tyr Glu Cys Lys His Thr Lys Ile Asn Pro Asp
                325                 330                 335

Tyr Asn Tyr Thr Asn Phe Asp Asn Phe Gly Trp Ser Phe Leu Ala Met
            340                 345                 350

Phe Arg Leu Met Thr Gln Asp Ser Trp Glu Lys Leu Tyr Gln Gln Thr
        355                 360                 365

Leu Arg Thr Thr Gly Leu Tyr Ser Val Phe Phe Phe Ile Val Val Ile
    370                 375                 380

Phe Leu Gly Ser Phe Tyr Leu Ile Asn Leu Thr Leu Ala Val Val Thr
385                 390                 395                 400

Met Ala Tyr Glu Glu Gln Asn Lys Asn Val Ala Ala Glu Ile Glu Ala
                405                 410                 415

Lys Glu Lys Met Phe Gln Glu Ala Gln Gln Leu Leu Lys Glu Glu Lys
            420                 425                 430

Glu Ala Leu Val Ala Met Gly Ile Asp Arg Ser Ser Leu Thr Ser Leu
        435                 440                 445

Glu Thr Ser Tyr Phe Thr Pro Lys Lys Arg Lys Leu Phe Gly Asn Lys
    450                 455                 460

Lys Arg Lys Ser Phe Phe Leu Arg Glu Ser Gly Lys Asp Gln Pro Pro
465                 470                 475                 480

Gly Ser Asp Ser Asp Glu Asp Cys Gln Lys Lys Pro Gln Leu Leu Glu
                485                 490                 495

Gln Thr Lys Arg Leu Ser Gln Asn Leu Ser Leu Asp His Phe Asp Glu
            500                 505                 510
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His Gly Asp Pro Leu Gln Arg Gln Arg Ala Leu Ser Ala Val Ser Ile
        515                 520                 525

Leu Thr Ile Thr Met Lys Glu Gln Glu Lys Ser Gln Glu Pro Cys Leu
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Pro Cys Gly Glu Asn Leu Ala Ser Lys Tyr Leu Val Trp Asn Cys Cys
545                 550                 555                 560

Pro Gln Trp Leu Cys Val Lys Lys Val Leu Arg Thr Val Met Thr Asp
                565                 570                 575

Pro Phe Thr Glu Leu Ala Ile Thr Ile Cys Ile Ile Ile Asn Thr Val
            580                 585                 590

Phe Leu Ala Met Glu His His Lys Met Glu Ala Ser Phe Glu Lys Met
        595                 600                 605

Leu Asn Ile Gly Asn Leu Val Phe Thr Ser Ile Phe Ile Ala Glu Met
    610                 615                 620

Cys Leu Lys Ile Ile Ala Leu Asp Pro Tyr His Tyr Phe Arg Arg Gly
625                 630                 635                 640

Trp Asn Ile Phe Asp Ser Ile Val Ala Leu Leu Ser Phe Ala Asp Val
                645                 650                 655

Met Asn Cys Val Leu Gln Lys Arg Ser Trp Pro Phe Leu Arg Ser Phe
            660                 665                 670

Arg Val Leu Arg Val Phe Lys Leu Ala Lys Ser Trp Pro Thr Leu Asn
        675                 680                 685

Thr Leu Ile Lys Ile Ile Gly Asn Ser Val Gly Ala Leu Gly Ser Leu
    690                 695                 700

Thr Val Val Leu Val Ile Val Ile Phe Ile Phe Ser Val Val Gly Met
705                 710                 715                 720

Gln Leu Phe Gly Arg Ser Phe Asn Ser Gln Lys Ser Pro Lys Leu Cys
                725                 730                 735

Asn Pro Thr Gly Pro Thr Val Ser Cys Leu Arg His Trp His Met Gly
            740                 745                 750

Asp Phe Trp His Ser Phe Leu Val Val Phe Arg Ile Leu Cys Gly Glu
        755                 760                 765

Trp Ile Glu Asn Met Trp Glu Cys Met Gln Glu Ala Asn Ala Ser Ser
    770                 775                 780

Ser Leu Cys Val Ile Val Phe Ile Leu Ile Thr Val Ile Gly Lys Leu
785                 790                 795                 800

Val Val Leu Asn Leu Phe Ile Ala Leu Leu Leu Asn Ser Phe Ser Asn
                805                 810                 815
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Glu Glu Arg Asn Gly Asn Leu Glu Gly Glu Ala Arg Lys Thr Lys Val
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Gln Leu Ala Leu Asp Arg Phe Arg Arg Ala Phe Cys Phe Val Arg His
835 840 845

Thr Leu Glu His Phe Cys His Lys Trp Cys Arg Lys Gln Asn Leu Pro
850 855 860

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885 890 895

Leu Gly Ile Leu Thr Ser Val Pro Lys Thr Leu Gly Val Arg His Asp
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Trp Thr Trp Leu Ala Pro Leu Ala Glu Glu Glu Asp Asp Val Glu Phe
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Ser Gly Glu Asp Asn Ala Gln Arg Ile Thr Gln Pro Glu Pro Glu Gln
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Gln Ala Tyr Glu Leu His Gln Glu Asn Lys Lys Pro Thr Ser Gln Arg
945 950 955 960

Val Gln Ser Val Glu Ile Asp Met Phe Ser Glu Asp Glu Pro His Leu
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Thr Ile Gln Asp Pro Arg Lys Lys Ser Asp Val Thr Ser Ile Leu Ser
980 985 990

Glu Cys Ser Thr Ile Asp Leu Gln Asp Gly Phe Gly Trp Leu Pro Glu
995 1000 1005

Met Val Pro Lys Lys Gln Pro Glu Arg Cys Leu Pro Lys Gly Phe Gly
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Cys Cys Phe Pro Cys Cys Ser Val Asp Lys Arg Lys Pro Pro Trp Val
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Trp Phe Glu Ser Phe Ile Ile Phe Val Ile Leu Leu Ser Ser Gly Ala
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Trp Ile Asn Gln Lys Val Asn Phe Asp Asn Val Gly Asn Ala Tyr Leu
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Ala Ala Val Asp Ser Thr Glu Lys Glu Gln Gln Pro Glu Phe Glu Ser
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Asn Ser Leu Gly Tyr Ile Tyr Phe Val Val Phe Ile Ile Phe Gly Ser
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Gln Lys Lys Tyr Tyr Asn Ala Met Lys Lys Leu Gly Ser Lys Lys Pro
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Gln Lys Pro Ile Pro Arg Pro Leu Asn Lys Cys Gln Gly Leu Val Phe
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Asp Ile Val Thr Ser Gln Ile Phe Asp Ile Ile Ile Ile Ser Leu Ile
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Ile Leu Asn Met Ile Ser Met Met Ala Glu Ser Tyr Asn Gln Pro Lys
1380 1385 1390

Ala Met Lys Ser Ile Leu Asp His Leu Asn Trp Val Phe Val Val Ile
1395 1400 1405

Phe Thr Leu Glu Cys Leu Ile Lys Ile Phe Ala Leu Arg Gln Tyr Tyr
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Phe Thr Asn Gly Trp Asn Leu Phe Asp Cys Val Val Val Leu Leu Ser
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                1445                1450                1455

Phe Pro Pro Thr Leu Phe Arg Ile Val Arg Leu Ala Arg Ile Gly Arg
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Ile Leu Arg Leu Val Arg Ala Ala Arg Gly Ile Arg Thr Leu Leu Phe
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Ala Leu Met Met Ser Leu Pro Ser Leu Phe Asn Ile Gly Leu Leu Leu
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Phe Leu Ile Met Phe Ile Tyr Ala Ile Leu Gly Met Asn Trp Phe Ser
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Lys Val Asn Pro Glu Ser Gly Ile Asp Asp Ile Phe Asn Phe Lys Thr
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Phe Ala Ser Ser Met Leu Cys Leu Phe Gln Ile Ser Thr Ser Ala Gly
            1540                1545                1550

Trp Asp Ser Leu Leu Ser Pro Met Leu Arg Ser Lys Glu Ser Cys Asn
        1555                1560                1565

Ser Ser Ser Glu Asn Cys His Leu Pro Gly Ile Ala Thr Ser Tyr Phe
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Val Ser Tyr Ile Ile Ile Ser Phe Leu Ile Val Val Asn Met Tyr Ile
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Ala Val Ile Leu Glu Asn Phe Asn Thr Ala Thr Glu Glu Ser Glu Asp
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Pro Leu Gly Glu Asp Asp Phe Asp Ile Phe Tyr Glu Val Trp Glu Lys
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Phe Asp Pro Glu Ala Thr Gln Phe Ile Lys Tyr Ser Ala Leu Ser Asp
        1635                1640                1645

Phe Ala Asp Ala Leu Pro Glu Pro Leu Arg Val Ala Lys Pro Asn Lys
    1650                1655                1660

Tyr Gln Phe Leu Val Met Asp Leu Pro Met Val Ser Glu Asp Arg Leu
1665                1670                1675                1680

His Cys Met Asp Ile Leu Phe Ala Phe Thr Ala Arg Val Leu Gly Gly
                1685                1690                1695

Ser Asp Gly Leu Asp Ser Met Lys Ala Met Met Glu Glu Lys Phe Met
            1700                1705                1710

Glu Ala Asn Pro Leu Lys Lys Leu Tyr Glu Pro Ile Val Thr Thr Thr
        1715                1720                1725
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Arg Lys Tyr Met Met Lys Val Thr Lys Gly Asp Gln Gly Asp Gln Asn
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/19342

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C07K 14/705; C12N 5/10, 15/12, 15/63

US CL :435/320.1, 325; 530/350; 536/23.5

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/320.1, 325; 530/350; 536/23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BRS, STN, MEDLINE, GENESEQ, PIR, SWISS-PROT, STREMBL, GENEMBL

search terms: sodium channel, tetrodotoxin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - A	WO 97/01577 A1 (UNIVERSITY COLLEGE LONDON) 16 January 1997, see especially pages 85-93.	1-6, 8-9, 22, 25 --- 7, 33-35

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 10 SEPTEMBER 2000	Date of mailing of the international search report 02 NOV 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MICHAEL PAK Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/19342

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ **Claims Nos.:**
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ **Claims Nos.:**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ **Claims Nos.:**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-9, 22, 25 , 33-35

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/19342

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-9, 22, 25, 33-35, drawn to an isolated nucleic acid molecule, a method of producing a transformed cell, a Na+ channel and a method of producing recombinant NaN protein.
Group II, claim(s) 10-14, drawn to a method to identify an agent that modulates the activity of the Na+ channel.
Group III, claim(s) 15, drawn to a method to identify an agent that modulates the transcription or translation of mRNA.
Group IV, claim(s) 16, drawn to a method to treat pain by administering an agent that modulates the activity of the Na+ channel.
Group V, claim(s) 17, drawn to a method to treat pain by administering an agent that modulates the transcription or translation of mRNA.
Group VI, claim(s) 18, drawn to an isolated nucleic acid that is antisense.
Group VII, claim(s) 19, drawn to a scintigraphic method to image loci of pain generation.
Group VIII, claim(s) 20, drawn to a method to identify tissues by detecting NaN.
Group IX, claim(s) 21, drawn to a method to identify tissues by detecting mRNA.
Group X, claim(s) 23-24, drawn to an antibody.
Group XI, claim(s) 26-27 and 36-37, drawn to a therapeutic composition comprising an agent and a method of treatment using the agent of claim 26.
Group XII, claim(s) 28, drawn to a method to screen candidate compound for use in treating pain.
Group XIII, claim(s)29-32, drawn to a chimeric NaN channel and the DNA encoding the chimeric NaN channel.

The inventions listed as Groups I-XIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features because the product of claim 1 is anticipated by UNIVERSITY COLLEGE LONDON (WO 97/01577A1 16 January 1997) and thus, does not share a special technical feature with any other group.

The products of Group VI, X-XI, and XIII does not share the same or corresponding special technical feature with Group I, because they are drawn to products having materially different structures and functions, and each defines a separate invention over the art.
The methods of Groups II-V, VII-IX, and XII do not share the same or corresponding special technical feature with Group I, because the methods have materially different process steps and are practiced for materially different purposes, and each defines a separate invention over the art.

Since Groups I-XIII do not share a special technical feature, unity of invention is lacking.